Vol. 13, No. 3 August, 1921

Medical Lib
SEP 28 1921

PARASITOLOGY

EDITED BY

GEORGE H. F. NUTTALL, M.D., Ph.D., Sc.D., F.R.S.

Quick Professor of Biology in the University of Cambridge

CAMBRIDGE UNIVERSITY PRESS

C. F. CLAY, MANAGER

LONDON: FETTER LANE, E.C. 4

ALSO

H. K. LEWIS AND CO., LTD., 136, GOWER STREET, LONDON, W.C. 1

WHELDON AND WESLEY, LTD., 28, ESSEX STREET, STRAND, LONDON, W.C. 2

CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENT FOR THE UNITED STATES AND CANADA)

BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.

TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

Fifteen Shillings net

[*Issued* 31 *August*, 1921]

The University of Chicago Press

The Anatomy of Woody Plants.

By E. C. JEFFREY, Professor of Plant Morphology in Harvard University. The author is easily the leading American Authority on the anatomy of plants. His book aims to describe, analyze, and discuss the anatomical structure of the most important families of plants. It does for the anatomy of plants what so many textbooks have done for the human anatomy. 306 figures and illustrations; x+478 pages, cloth; 31*s* net.

The Evolution of Sex in Plants. (*The University of Chicago Science Series.*)

By JOHN M. COULTER, Head of the Department of Botany, The University of Chicago. In this work the prevalence of a sexual reproduction among plants is emphasized, the author showing that in many of the lower forms sexual reproduction is unknown. Sexual reproduction is described historically as the last method of reproduction attained among plants, and as not replacing but as being added to earlier methods. Thus we have variation, and in sex reproduction extreme individuality also. The more detailed parts of the discussion are illustrated by cuts and drawings introduced into the text. x+140 pages, 12mo, cloth; 8*s* 3*d* net.

Individuality in Organisms. (*The University of Chicago Science Series.*)

By CHARLES MANNING CHILD, Associate Professor of Zoology in the University of Chicago. Professor Child's work is an attempt to state, and to present evidence in support of, a conception of the nature of organic individuality which the author has developed as a result of fifteen years of investigation of the processes of reproduction and development in the lower animals. In these forms, organic individuality appears in relatively simple terms, and it is here that we must look for the key to the problem of individuality in the higher animals and man.

The author has addressed himself with remarkable success to showing the wide range of applicability of the conception of individuality of various fields, with the result that the book appeals not only to the physiologist and to the botanist, but also to the neurologist, to the psychologist, and even to the sociologist. x+212 pages, small 12mo, cloth; 10*s* net.

Sold in the British Empire (except Canada) by

Cambridge University Press, Fetter Lane, London, E.C. 4

Cambridge University Press

AN INTRODUCTION TO THE STUDY OF CYTOLOGY. By L. DONCASTER, Sc.D., F.R.S., Derby Professor of Zoology in the University of Liverpool. Demy 8vo. With 24 plates and 31 text-figures. 21*s* net.

GROWTH AND FORM. By D'ARCY WENTWORTH THOMSON, C.B., D.Litt., F.R.S., Professor of Natural History in the University of St Andrews; Scientific Member of the Fishery Board for Scotland. Demy 8vo. With 408 illustrations. 24*s* net.

ZOOLOGY. An Elementary Textbook. By Sir A. E. SHIPLEY, G.B.E., Sc.D., F.R.S., and E. W. MACBRIDE, D.Sc., F.R.S. Fourth edition. Demy 8vo. With 360 illustrations. 20*s* net.

THE FLEA. By H. RUSSELL, B.A., F.Z.S., M.B.O.U. Roy. 16mo. With 9 illustrations. Cloth, 2*s* 6*d*. Limp Lambskin, 3*s*. Cambridge Manuals of Science and Literature.

THE CAUSES OF TUBERCULOSIS. Together with some Account of the Prevalence and Distribution of the Disease. By L. COBBETT, M.D., F.R.C.S. With 23 plates and 8 diagrams. 23*s* net.

INDUSTRIAL COLONIES AND VILLAGE SETTLEMENTS FOR THE CONSUMPTIVE. By Sir GERMAN WOODHEAD, K.B.E., V.D., M.D., LL.D., and P. C. VARRIER-JONES, M.R.C.S., L.R.C.P., Tuberculosis Officer for the County of Cambridge. With a Preface by Sir CLIFFORD ALLBUTT. Demy 8vo. Paper covers, 9*s* net; cloth, 10*s* 6*d* net.

Cambridge University Press, Fetter Lane, London, E.C. 4; C. F. Clay, Manager

KARL ASMUND RUDOLPHI
1771—1832

PORTRAITS

Separate copies of portraits appearing in *Parasitology* may be obtained on application to the Secretary, Quick Laboratory, The Museums, Cambridge.

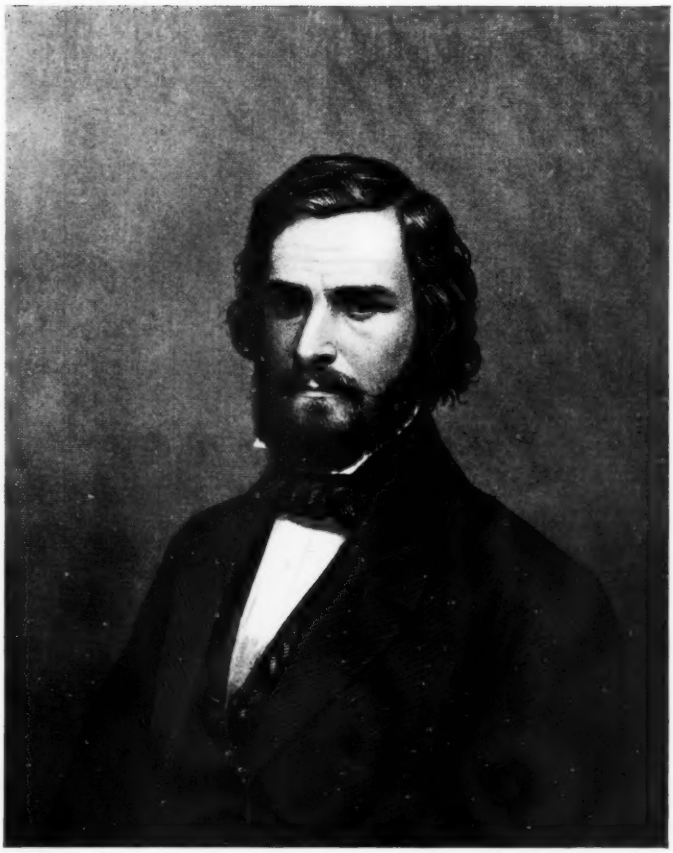
The price of each Portrait-plate is one shilling, post free. Payments should accompany orders.

The last number of each volume will contain brief notes bearing on the subjects of the portraits.

G. H. F. NUTTALL.

JOHANNES MÜLLER
1801—1858

Separate copies may be obtained from the University Press, Cambridge



JOSEPH LEIDY

1823—1891

Separate copies may be obtained from the University Press, Cambridge

VOLUME XIII AUGUST, 1921 No. 3

ON A NEW CESTODE FROM THE POUCHED RAT, *CRICETOMYS GAMBIANUM*.

BY F. J. MEGGITT, M.Sc., PH.D.,

Assistant Helminthologist, University of Birmingham.

(*From the Research Laboratory in Agricultural Zoology, University of Birmingham.*)

(With Plate IX.)

THE material of the species to be described was found in a small collection of parasites from Zanzibar, placed at my disposal by Professor G. H. F. Nuttall, F.R.S., to whom I wish to express my thanks. As only a few cestodes have hitherto been recorded from East Africa and its helminth fauna is therefore largely unknown, a list of the forms identified is given at the end of the paper. I desire to acknowledge my indebtedness to the Birmingham Natural History and Philosophical Society for a grant towards the expense of preparing the plates, both in this and the preceding paper (*Parasitology*, XIII. No. 1, March 1921, p. 1).

The specimens of the new species were obtained from the duodenum of the pouched rat (*Cricetomys gambianum*), and consist of five strobili, two with scoleces, and numerous fragments. The scolex (Pl. IX, fig. 3) is 0·5 mm. long × 0·66–0·75 mm. diameter, is furnished with a small apical depression devoid of any muscular structure and with four suckers, two dorsal and two ventral. These lie in pockets inside the scolex, opening to the exterior by a small orifice. The musculature of the sucker does not completely fill the pocket but leaves vacant near the opening a small non-muscular circle, sharply crinkled round the edges. Considerable difference of opinion exists upon the position of the suckers in this genus. According to Klaptocz (1906, 139) in *I. gondokorensis* they are to be found inside the scolex at the bottom of long funnel-shaped pockets: this is true of *I. hyracis*, *I. interpositus* and *I. setti* according to von Janicki (1910, 394) and is cautiously confirmed by Bischoff (1913, 248) for his own species; "Auch alle von mir untersuchten Arten von *Inermicapsifer* wiesen teils mehr, teils weniger deutlich diese typischen Saugnapftaschen auf." On the other hand Beddard (1912, 578) states: "Nor could I detect anything peculiar in the structure of the suckers, which, according to Janicki and others, are remarkable for a funnel-like ingrowth leading to the actual sucker, which thus lies at the bottom of a depression. It is true that

in the present species, as in many tapeworms of the group Tetracotylea which I have examined, the sucker does not lie externally on the scolex, but is covered by a layer of body-wall which is only interrupted at the orifice of the sucker. The free edge of this, when depressed towards the interior of the sucker, is doubtless funnel-shaped and would give rise to the appearances represented by Janicki. Perhaps, however, there is some divergence from the normal condition of the suckers in the members of the genus referred to by him, which I certainly have not found in the species with which I am at present concerned." The suckers figured by von Linstow (1906, Pl. XIII, fig. 9) for *Z. remota* and Pagenstecher (1878, Pl. X, fig. 2) for *I. criticus* are obviously on the surface of the scolex in the usual Cyclophyllidean position. In *Z. muricola*, according to Baylis (1915, 41), they occupy an intermediate position, "they are sunk somewhat deeply in the substances of the head, and their orifices are flush with the surface." My own observations confirm the statement of Beddard just quoted. The suckers are distinctly under the surface of the scolex but not at the bottom of the funnels and they thus present a very different appearance to the usual Cyclophyllidean sucker with a rim of musculature on the external surface of the head. The greatest diameter of the suckers observed was 0·2 mm. Behind them the scolex bulged to a considerable extent, its posterior limit being twice the diameter of the long unsegmented neck which follows it. The proglottides are broader than long for the greater part of the strobili, the more terminal ones being approximately square.

The musculature of each proglottis (Pl. IX, figs. 5–7) is exceedingly weak, consisting of only two layers, one transverse and one longitudinal. The latter is composed of a number of isolated fibres—only occasionally do two or three of them become aggregated together into a bundle—scattered irregularly throughout the cortical parenchyma. Internally to them lie several strands of transverse muscles forming a band enclosing the genital organs and medullary parenchyma.

The excretory system consists of the usual four longitudinal vessels, two on each side of the proglottis—these being arranged laterally to each other instead of dorsally and ventrally. The innermost is large and thin-walled and communicates with its fellow of the opposite side by a large transverse commissure at the posterior margin of the proglottis. It also gives off here an outer vessel the branches of which, running anteriorly, anastomose with each other and with the parent stem, forming a small excretory plexus. The outer longitudinal vessel is small and thick-walled, gives off no lateral branches, and does not communicate with that on the opposite side by any transverse commissures.

The genital pore is unilateral, nearly at the anterior quarter of the proglottis margin (Pl. IX, fig. 4). A small and narrow genital cloaca is present and into it open anteriorly and posteriorly the cirrus-sac and vagina respectively. The genital ducts pass between the excretory vessels and—assuming the larger excretory vessel to correspond to the ventral vessel usually present and the smaller to the dorsal—dorsal to the nerve.

The cirrus-sac is small and pear-shaped, the greatest diameter being internally, 0·14–0·16 mm. long × 0·04 mm. dia., and does not reach the nerve. The cirrus is unarmed and opens into a small straight vesicula seminalis which fills the remainder of the sac. Outside the cirrus-sac the vas deferens forms a bundle of loose coils extending as far as the inner longitudinal excretory vessel, there breaking up into the vasa efferentia. The testes are numerous, 120–130 in each proglottis, and form two groups, separated only for a small space by the female organs. On the aporal side they number 106—the number in the proglottides counted was remarkably constant—and are arranged in the form of a wedge with the longer axis lying transversely across the strobilus and the testes more numerous laterally than medianly. They extend as far anteriorly as the margin of the preceding proglottis. Half-way across they diminish to two rows along the posterior border and to a single row at their junction with the female organs. The poral group consists of from 15–20 testes arranged in a double row along the posterior margin and not extending further anteriorly than the vagina. Like the aporal group, these narrow to a single row on reaching the female organs. The two groups are separated only by the diameter of the yolk-gland, appearing under a low magnification to be a continuous row along the posterior margin, the yolk-gland apparently being the connecting link. Laterally both groups extend beyond the two excretory vessels, but do not pass the nerve. Dorso-ventrally the testes lie in a single layer, but in a few places this may be doubled.

The female glands lie porally, a quarter of the distance across the proglottis, internally to both poral excretory vessels. The ovary lies on the ventral surface immediately median to the inner excretory vessel, half-way between the anterior and posterior margins. It is small and straggling, the lobes being long and slender and only slightly connected with one another. Posteriorly and aporally to it is the yolk-gland, also lobed but with blunter processes. The distal portion of the vagina is thick-walled and surrounded with gland cells. On crossing the lateral nerve, this portion communicates by an exceedingly fine duct with a large thin-walled receptaculum seminalis full of spermatozoa, constricted in places, and extending almost to the ovary. Dorsal to the latter is the shell-gland. A definite but ephemeral uterus is developed. The oviduct, after passing through the shell-gland, runs anteriorly and becomes a thick-walled glandular tube with an exceedingly narrow lumen. On arriving dorsally to the receptaculum seminis the lumen increases, the walls become studded with nuclei and the duct, curving internally, opens into the uterus. This latter is a thin-walled sac with a narrow dorso-ventral diameter occupying only one-fifth of the proglottis but extending laterally beyond both excretory vessels and reaching the nerve. In it the eggs are arranged in a single layer: occasionally one or two become pushed one on top of the other but there is never any outgrowth as figured by Janicki (1910, Pl. XII, fig. 13) for *Inermicapsifer hyracis* (Pall.). The degeneration of the uterus and the formation of the egg-capsules agree very well with the account of Janicki just

mentioned. There is a tendency for the eggs in the uterus to become isolated each in separate cavities, and as the proglottis matures this process continues until all the eggs lie in a single dorso-ventral row, each in its own egg-capsule formed from the walls of the disappearing uterus (Pl. IX, fig. 5). These capsules become aggregated together in groups of 9–11 and surrounded by a fibrous, rather spongy modification of the body parenchyma; this forms a distinct sheath round each group and also penetrates in between the individual capsules. Each group of eggs thus becomes completely separated from its neighbours (Pl. IX, fig. 6). In a transverse section there are approximately 16 of these capsules, extending on both sides past the excretory vessels and nearly reaching the nerve: dorso-ventrally they occupy nearly all the space between the two opposite layers of transverse muscle.

Between these capsules there is still left a certain amount of medullary parenchyma, but as growth proceeds this completely disappears, the egg-capsules, becoming polygonal by compression, occupy all the space bounded by the transverse muscles and form a double dorso-ventral layer instead of a single one (Pl. IX, fig. 7). Each capsule has now a definite delimiting membrane externally and consists of two portions: an outer fibrous spongy mass, and an inner granular non-nucleated matrix in which are imbedded the eggs. I could not find any traces of the second layer figured by Baylis (1915, fig. 6). In each proglottis, now approximately square, are 40–50 egg-capsules each containing 9–11 eggs and filling all the space between the two longitudinal nerves in one direction and between the anterior and posterior margins of the proglottis in the other.

The possession of an unarmed scolex and the absence of a persistent uterus place this worm in the sub-family *Linstowinae* Fuhrmann 1907. This includes up to the present six genera, two of which, *Linstowia* Zschokke 1898 and *Oochoristica* Lühe 1898, are definitely separated from the form under discussion by the position of the female organs in the median portion of the proglottis. The third genus, *Hyracotaenia* Beddard 1912, by the absence of a persistent uterus (a character which should remove the genus from this sub-family) can therefore be dismissed. The other three genera, *Inermicapsifer* v. Janicki 1910, *Zschokkeella* Fuhrmann 1902 and *Thysanotaenia* Beddard 1911 are closely allied and, up to the present, insufficiently separated from each other.

The only characters distinguishing *Thysanotaenia* appear to be the poor development of the excretory system, the more median position of the female organs and the larger size of the cirrus-sac. The variability of the excretory system is often very great in allied species; in *Oochoristica crassiceps* Baylis (1920, p. 293) found no excretory plexus nor any transverse commissures; on the other hand in *O. wagneri* v. Janicki the excretory system is well developed. Rudin (1917, p. 317) states: "Ganz ausserordentlich sind die verschiedenen Formen der Ausbildung, die das Excretionssystem, nicht nur innerhalb der Genera *Ophiotaenia* und *Acanthotaenia*, sondern im allgemeinen innerhalb der

Familie der Ichthyotaenien, aufweist," proceeding then to elaborate the differences between the species of the same genus. The position of the female glands in the species of a genus is not constant. In the genus *Davainea* Blanchard 1891 they customarily lie on the median axis of the strobilus, but in *D. leptotrachela* Hungerbühler 1910 they are poral in position, nearly touching the longitudinal excretory vessels, and again in *D. tetragona* (Molin 1858) and *D. echinobothrida* (Mégnin 1880) are distinctly poral. With regard to the size of the cirrus-sac, in his account of the type species, *T. lemuris*, Beddard (1911 *a*) gives neither measurements nor a statement of its extent, merely stating (1912, p. 607) "Cirrus-sac rather large," a vague statement unsuitable for use as a generic character. Neither do the three characters taken in combination assume sufficient importance to be of generic rank. From Beddard's description therefore, unless subsequently further distinctive characters be forthcoming, I would suggest the placing of *T. lemuris* in the genus *Zschokkeella*,—the egg-capsules being formed altogether independently of the uterus (1911, p. 1000)—and the suppression of the genus *Thysanotaenia*.

For the identification of the two remaining genera Beddard (1912, p. 607) has provided a somewhat unsatisfactory key. He places *I. hyracis* (Pall.)[1] in the genus *Zschokkeella* and then distinguishes between the two genera by crediting *Inermicapsifer* with posterior genital pores, two groups of testes entirely separated from each other, a vas deferens together with a seminal network, and the absence of a well-developed uterus, and *Zschokkeella* with median genital pores, a continuous row of testes, the absence of a vesicula seminalis (and presumably of a seminal network?) and the presence of a well-developed uterus. With regard to the first character, the disposition of the testes, there is every variation between the type of *I. setti* v. Janicki 1910 with the testes in two clearly separated groups, through *Z. gambianum* Beddard 1911 (1912, p. 582), where three testes form a connecting link between the two groups to *I. hyracis* (Pall.) where they are arranged in a continuous band along the posterior border of the segment, and *I. paronae* Bischoff 1912, where they are equally distributed throughout the whole proglottis, extending anteriorly to the genital ducts. For *Zschokkeella muricola* Baylis (1915, p. 47) states: "The testes are divisible into two groups; but they so nearly form a complete series across the segment (except where interrupted by the female organs) that it is difficult to say whether their arrangement is more like that seen in *Inermicapsifer* or that in *Zschokkeella*. In this respect the species seems to be intermediate between the two genera." A character so liable to variation as this cannot be considered adequate for the discrimination of genera. The further distinction drawn by means of the vesicula seminalis is inaccurate. Fuhrmann (1902, p. 139) for *Z. linstowi*, the type species

[1] *Taenia hyracis* Pallas 1767 was selected by von Janicki (1910) for the type species of his genus *Inermicapsifer*. If this species be removed to the genus *Zschokkeella* (Fuhrmann 1901), the name *Inermicapsifer* falls into synonymy with *Zschokkeella* and Beddard's first group, with testes in two distinct groups, should be renamed.

where, according to Beddard's table, it should be absent, states: "Am inner Ende der schwach muskulösen Penistasche liegt eine kleinere Vesicula seminalis." On the other hand, Baylis (1915, p. 43) found it absent in *Z. muricola* while, with the exception of *I. capensis* Beddard 1912 and the new species just described, it is absent or, being unmentioned, presumably absent in all the nominal species of *Inermicapsifer*, whether with a continuous band of testes and therefore referable to *Zschokkeella*, or with two groups and therefore referable to *Inermicapsifer*. The position of the genital pore, the third character, is anterior in *I. parvulus* Bischoff 1912, and posterior in *I. setti* v. Janicki 1910—both with two separate groups of testes—and anterior in *Z. remota* v. Linstow 1906 and posterior in *I. parona* Bischoff 1912—both with a continuous band of testes. The last character in the key, uterus well or little developed, is a comparative one and therefore suffers from vagueness. In *I. hyracis* the uterus is stated Janicki (1910, p. 381) to extend between the excretory vessels as a broad sack with outgrowths, disappearing to form the walls of the primary egg-capsules: in *I. capensis* the oviduct is stated (Beddard 1912, 586), to be "quite short and ends more or less abruptly in a strand of condensed parenchymal tissue." In *Z. gambianum* (Beddard 1911, p. 659) the uterus is "not much more than a transversely running tube extending nearly right across the proglottis in which the eggs occur, but with which the uterus never appears to be stuffed. I could find no outgrowths of this centrally-placed uterus, and there was certainly nothing in the nature of a reticular formation of its cavity." Other investigators are content merely to state, "Der Uterus, der sich auf jüngerem Stadium unregelmässig zwischen die Hoden einscheibt, löst sich etwa im letzten Viertel der Strobila sehr rasch in Eikapseln auf" (Bischoff 1913, p. 238): "The uterus persists through about 28 segments, but after a short time appears to break down altogether, and the ova are seen to be scattered among the parenchyma" (Baylis 1915, p. 44): and "Der Uterus bildet angangs ein median verlaufendes, leicht gewelltes Rohr, das beiderseits bis an die Längsnerven reicht. Seine Wandung verscheint bald und werden die Eier ins Parenchyme gestossen" (Fuhrmann 1902, 140). *I. capensis* is the only species which can thus definitely be placed in the genus *Inermicapsifer*, the remaining 17 species falling into the genus *Zschokkeella* which then becomes a rather heterogeneous assemblage, for the testes may be continuous (*I. hyracis*) or in two groups (*I. setti*), a vesicula seminalis may be present (*Z. linstowi*) or absent (*Z. muricola*), and the genital pore posterior (*I. paronae*), or median (*I. interpositus* v. Janicki 1910), or anterior (*Z. muricola*) and there may be almost any permutation and combination of these characters. From the above facts it may be seen that it is impossible to use Beddard's definition of the two genera unless it is prepared to reserve the genus *Inermicapsifer* for *I. capensis* alone and make *Zschokkeella* a dumping ground for all the other species.

Von Janicki (1910, p. 394) distinguishes his genus from *Zschokkeella* by the following points: eggs in parenchymatous capsules and not merely in "Binde-

gewebe," suckers sunk in pockets, not on the surface of the scolex; greater extension internally of the ventral excretory system, the greater development of medullary parenchyma and of parenchymatous muscles; the greater length of the terminal proglottides; the restriction of the testes to the more posterior portion of the proglottis; and the position of the female organs median to the excretory system. I am in entire agreement with Beddard's criticism of this (1912, p. 606): the points mentioned are either trivial or not in accordance with the facts.

There only remains the question of the formation of the egg-capsules. For *I. hyracis* (p. 381), *I. interpositus* (p. 386) and *I. setti* (p. 388) v. Janicki asserts that the eggs are first enclosed singly in capsules formed from the uterine walls and only secondarily in capsules formed of modified medullary parenchyma; after this, the primary capsule wall disintegrates—a process similar to that described by him for *D. polycalceola* (1902, 258). This account is borne out by Bischoff for *I. paronae* (1913, 238) and *I. lopas* (p. 244) and confirmed by the behaviour of the uterus in the species described at the commencement of the paper. On the other hand Beddard (1912, 587) asserts that in *I. capensis* the uterus is never developed as a definite tube with a lumen and that therefore the egg-capsules can have no connection with it, and for *Z. gambianum* states (1911, 659): "Gradually the cavity of the uterus appeared, as it were, to dry up and the eggs were found—to continue the simile—stranded in the tissue of the body." Baylis again for *Z. muricola* states (1915, 45): "There seems to be no ground for supposing that the capsules in which the eggs are enclosed, whether they are to be regarded as 'parauterine organs' or not, are derived from the uterus." In his definition of *Zschokkeella* Fuhrmann states (1908, 40): "Uterus löst sich in Eikapseln auf," but on turning to his description of the type species (1901, 761) he there says: "Der Uterus löst sich auf, wie dies für das Genus *Linstowia* characteristisch ist, und kommen die Eier ins Parenchyme eingebettet zu liegen"; and (1902, 140): "Uterus verliert seine Wandung bald und die Eier treten ins Parenchyme über." (See also the quotation already given.) From these latter descriptions it does not appear that Fuhrmann wished to imply that the eggs are enclosed in capsules formed directly from the uterine walls, but that, on the contrary, they are deposited in the parenchyma and the capsules subsequently form around them, a process similar to that described by Beddard for *I. capensis* and *Z. gambianum* and in direct contradiction to Janicki's account of *I. hyracis*, *I. interpositus* and *I. setti*. This point seems to allow of a distinction being drawn between the two genera and I would suggest that the generic diagnoses be altered into "Eggs at first enclosed singly in capsules with walls derived from the uterus and only secondarily in capsules with walls of modified parenchyma" for *Inermicapsifer* and "Eggs enclosed singly, or in groups, in capsules in the formation of which the uterus has no part" for *Zschokkeella*. This would place definitely in *Inermicapsifer* the species *I. hyracis* (Pallas 1767), *I. setti* v. Janicki 1910, *I. interpositus* v. Janicki 1910, *I. lopas* Bischoff 1912

	Suckers	Genital pore	Testes	Vesicula seminalis	Ovary	Uterus	Formation of egg capsules	Number of egg capsules	Number of eggs per egg capsule	Host
Inermicapsifer										
I. abyssinicus Bischoff 1912	As in *I. interpositus*	In centre of proglottis margin	50–60, as in *I. interpositus*	—	—	—	—	24–26	8–10	*Procavia* sp.
I. apospamation Bischoff 1912	At bottom of pockets	In centre of proglottis margin	In two unequal clearly separated groups	—	Immediately median to poral (ventral?) excretory vessel	—	—	18–24	7	*Procavia* sp.
I. capensis Beddard 1912	Beneath surface of scolex	In posterior portion of proglottis margin	In two unequal clearly separated groups	Present	Median to both poral excretory vessels	Represented by condensed branched parenchymal tissue	Uterus has no part in their formation	—	—	*Procavia capensis* Schreber
I. criticus (Pagenstecher 1878)	On surface of scolex	In centre of proglottis margin	In a continuous posterior band?	—	—	—	—	180	—	*Procavia capensis* Schreber
I. gondokorensis Klaptocz 1906	At bottom of pockets	—	—	—	—	—	—	—	—	*Procavia slattini* Sassi
I. hyracis (Pallas 1767)	At bottom of pockets	In centre of proglottis margin	100–110, in a continuous posterior band	Absent	Dorsal to poral ventral excretory vessel	Well-developed sac containing eggs	Uterus forms primary capsule	80–100	4–5	*Procavia* sp.
I. interpositus v. Janicki 1910	At bottom of pockets	In centre of proglottis margin	80, in two continuous posterior rows	—	—	As in *I. hyracis*	As in *I. hyracis*	75	5–7	*Procavia* sp.
I. lopas Bischoff 1912	At bottom of pockets	In posterior portion of proglottis margin	60, in two clearly separated groups	—	Immediately median to poral (ventral?) excretory vessel	—	As in *I. hyracis*	12–15	12–16	*Procavia* sp.
I. pagenstecheri (Setti 1897)	On surface of scolex	In posterior portion of proglottis margin	Scattered throughout the entire proglottis	—	Poral	—	—	70–80	8–10	*Procavia* sp.
I. paronae Bischoff 1912	At bottom of pockets	In posterior portion of proglottis margin	Scattered throughout the entire proglottis	—	Median to poral ventral excretory vessel	As in *I. hyracis*	As in *I. hyracis*	30–50	6	*Procavia* sp.

I. parvulus Bischoff 1912	At bottom of pockets	In anterior portion of proglottis margin	20, in two unequal clearly separated groups	—	—	—	—	18–24	5–7	*Procavia* sp.
I. prionodes Bischoff 1912	At bottom of pockets	In posterior portion of proglottis margin	40, in a small layer a little posterior to the centre of the proglottis	—	Median to poral (ventral?) excretory vessel	—	—	26–28	5	*Procavia* sp.
I. setti v. Janicki 1910	At bottom of pockets	In posterior portion of proglottis margin	55, in two clearly separated groups	—	—	As in *I. hyracis*	As in *I. hyracis*	20	15	*Procavia* sp.
I. zanzibarensis n.sp.	Beneath surface of scolex	In anterior portion of proglottis margin	120–130, in two posterior indistinctly separated groups	Present	Median to poral ventral excretory vessel	Poorly developed	As in *I. hyracis*	40–50	10	*Cricetomys gambianum* Eth.
I. sp. v. Janicki 1910	Beneath surface of scolex	—	—	—	—	—	—	—	—	*Procavia syriaca* Ehrbg.
Zschokkeella										
Z. gambianum Beddard 1911	Beneath surface of scolex	In centre of proglottis margin	In two posterior clearly separated groups	Absent	Double each side the poral ventral excretory vessel	Transverse tube devoid of eggs	Uterus has no part in their formation	—	—	*Cricetomys gambianum* Eth.
Z. linstowi (Parona 1885)	On surface of scolex	In anterior portion of proglottis margin	140, in two lateral groups united by a posterior row	Present	Between the two poral excretory vessels	Transverse tube	—	—	—	*Numida ptilorhyncha* Licht.
Z. muricola Baylis 1920	Beneath surface of scolex	In anterior portion of proglottis margin	In two posterior practically continuous groups	Absent	—	Oblong sac	Uterus has no part in their formation	—	20	*Epimys rattus*
Z. remota v. Linstow 1905	On surface of scolex	In anterior portion of proglottis margin, slightly ventral	In a continuous band?	—	Median	—	—	—	—	*Cercopithecus pyrrhonotus* Ehrenb.
Thysanotaenia										
T. lemuris Beddard 1911	Beneath surface of scolex	In anterior portion of proglottis margin	In a continuous posterior band	Present	Anterior, slightly poral	Absent	—	—	3–4	*Lemur macaco*

and *I. paronae* Bischoff 1912, and in *Zschokkeella* the species *Z. linstowi* (Parona 1900), *Z. muricola* Baylis 1915, *Z. gambianum* (Beddard 1911), *I. capensis* Beddard 1912, and *Th. lemuris* Beddard 1911. In the remaining species the behaviour of the uterus has not been described and therefore they cannot be ascribed to either genus. A difference in the formation of the egg-capsules, as Beddard has pointed out, is hardly sufficient to distinguish two genera but, failing this, *Inermicapsifer* must fall into synonymy with *Zschokkeella* as no other character exists by which they can be distinguished one from the other. Until, therefore, a more detailed account of the behaviour of the uterus throughout the group be published it seems best to leave the two genera separated by this difference.

In accordance with this decision the new species described at the commencement of the paper (p. 193) belongs to the genus *Inermicapsifer* and by means of the table on pp. 200–201 can be distinguished from the other species. I propose for it therefore the name *Inermicapsifer zanzibarensis* n.sp.

Dipylidium oerley von Rátz, 1900.

The material of this species from the cat consists of three strobili and a few isolated proglottides. The greatest length observed was 40 mm. and the greatest breadth 1 mm. A scolex was not present.

The musculature of the mature proglottides (Pl. IX, fig. 1) in a transverse section consists of three longitudinal muscle bands, each bounded internally by transverse muscles. The innermost longitudinal muscle layer is composed of numerous small bundles, clearly separated one from the other, and formed by the aggregation of small fibres into groups of from 2 to 5: this layer, while distinct in the more posterior portion of the strobilus, becomes less marked anteriorly, until in proglottides with feebly developed male organs it entirely disappears. The layer external to it is wider and more continuous, the bundles are larger and formed of more fibres (approximately 20) and, together with the third layer, it is persistent throughout the proglottis. The outermost longitudinal muscle band is composed of numerous muscle fibres, aggregated into bundles of 5–8 internally and continuing as isolated strands nearly to the sub-cuticula. The transverse muscle layers are very irregular and vary greatly in appearance, occasionally disappearing altogether.

The excretory system in mature proglottides consists of a single large longitudinal vessel on each side, the two vessels communicating by the usual transverse commissure at the posterior end of each segment.

The genital pore is usually situated in a conspicuous depression in the anterior half of the proglottis margin; occasionally the depression is partially evaginated, becoming a small protuberance. The genital ducts pass dorsal to both excretory vessels and to the nerve. The cirrus-sac is 0·21 mm. long × 0·08 mm. diameter and extends just past the excretory vessels. Its musculature is very feeble and special retractor muscles are absent. The cirrus is

coiled and strengthened by annular thickenings similar to those in the protoxylem of Dicotyledons. When the cirrus lies inside the sac, these thickenings are on the exterior of its walls and project into the cavity of the sac. The space between the coils of the vas deferens is filled with deeply staining cellular tissue. The testes are over 100 and fill the whole proglottis except for the female organs and the short strip occupied by the genital ducts; this latter in stained specimens stands out as a prominent clear space stretching completely across the proglottis.

The eggs in mature segments lie in egg-sacs extending laterally past the excretory vessels. Each sac contains 8–14 eggs. In all other respects the form seen agreed with Hall's description (1920, 63).

LIST OF SPECIES FROM ZANZIBAR.

Anoplocephala perfoliata (Goeze 1782). Horse.
Dipylidium chyzeri von Rátz 1897. Domestic cat.
Dipylidium oerley von Rátz 1900. Domestic cat.
Inermicapsifer zanzibarensis n.sp. Pouched rat (*Cricetomys gambianum* Eth).
Stilesia globipunctata (Rivolta 1874). Domestic goat.
Stilesia hepatica Wolffhügel 1903. Sheep.
Stilesia vittata (Railliet 1896). Camel (*Camelus dromedarius* L.).
Taenia hydatigena (Pallas 1766). Dog.
Taenia taeniaeformis (Batsch 1786). Cat.

REFERENCES.

BAYLIS, H. A. (1915). A new Cestode of the genus *Zschokkeella*. *Ann. Mag. Nat. Hist.* ser. 8, XVI. 40–50.

—— (1920). Notes on some parasitic worms from East Africa. *Ibid.* ser. 9, VI. 283–295.

BEDDARD, F. E. (1911). On some mammalian Cestoidea. *Proc. Zool. Soc.* 626–660.

—— (1911 *a*). On two new genera of Cestodes from mammals. *Ibid.* 994–1018.

—— (1912). On a species of *Inermicapsifer* from the Hyrax, and on the genera *Zschokkeella*, *Thysanotaenia* and *Hyracotaenia*. *Ibid.* 576–607.

BISCHOFF, C. R. (1913). Cestoden aus Hyrax. *Rev. suisse Zool.* XXI. 225–284.

FUHRMANN, O. (1901). Bemerkungen über einige neuere Vogelcestoden. *Centrbl. Bakt.* Abt. 1, Orig. XXIX. 757–763.

—— (1902). Die Anoplocephaliden der Vögel. *Ibid.* XXXII. 122–147.

—— (1908). Die Cestoden der Vögel. *Zool. Jahrb.* Suppl. X.

HALL, M. C. (1920). The adult taenioid cestodes of dogs and cats and of related carnivores in North America. *Proc. U.S. Nat. Mus.* LV. 1–94.

JANICKI, C. v. (1902). Ueber zwei neue Arten des Genus *Davainea* aus Celebensischen Säugern. *Arch. Parasitol.* VI. 257–292.

—— (1910). Die Cestoden aus *Procavia*. *Jen. Denkschr.* XVI. 373–396.

LINSTOW, O. v. (1906). Helminthen aus Ceylon und aus arktischen Breiten. *Zeitschr. f. wiss. Zool.* LXXXII. 182–193.

PAGENSTECHER, H. A. (1878). Zur Naturgeschichte der Cestoden. *Ibid.* XXX. 171–193.

RUDIN, E. (1917). Die Ichthyotaenien der Reptilien. *Rev. suisse Zool.* XXV. 179–381.

DESCRIPTION OF PLATE IX.

The following letters apply to all the figures: *c.s.*, cirrus-sac; *d.exc.*, dorsal excretory vessel; *d.v.*, dorso-ventral muscles; *e.*, egg; *e.c.*, egg capsule; *l.m.*, *l.'m.'*, *l.''m.''*, longitudinal muscles; *ov.*, ovary; *r.s.*, receptaculum seminis; *t.*, testes; *t.m.*, *t.'m.'*, *t.''m.''*, transverse muscles; *u.*, uterus; *u'.*, remains of uterine wall; *v.exc.*, ventral excretory vessel; *y.g.*, yolk-gland.

Figs. 1–2. *Dipylidium oerley* von Rátz.

Fig. 1. Musculature of mature proglottis.
Fig. 2. Musculature of proglottis containing egg capsules.

Figs. 3–7. *Inermicapsifer zanzibarensis* n.sp.

Fig. 3. Scolex.
Fig. 4. Mature proglottis.
Fig. 5. Formation of egg capsules, first stage.
Fig. 6. Formation of egg capsules, second stage.
Fig. 7. Formation of egg capsules, third stage.

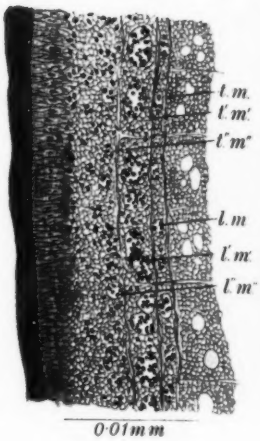


Fig. 1

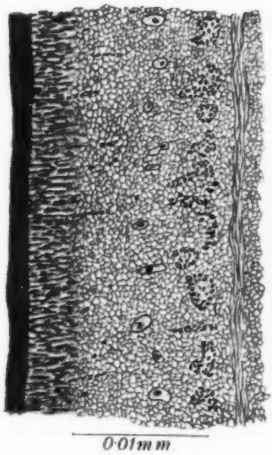


Fig. 2

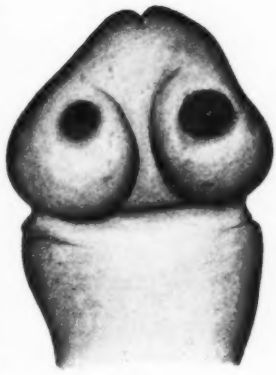


Fig. 3

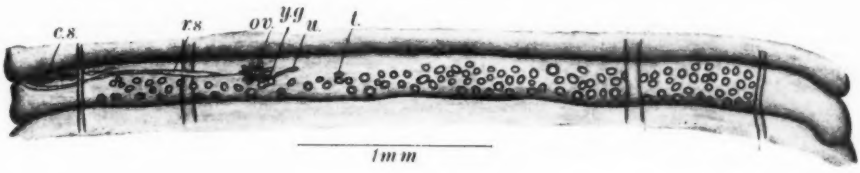


Fig. 4

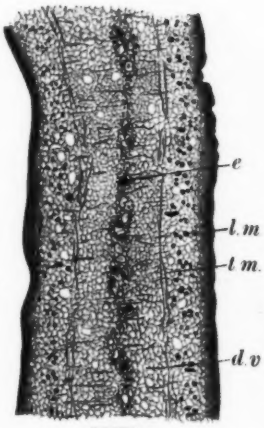


Fig. 5

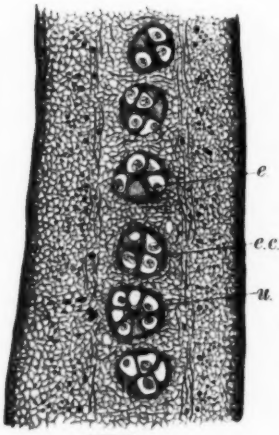


Fig. 6

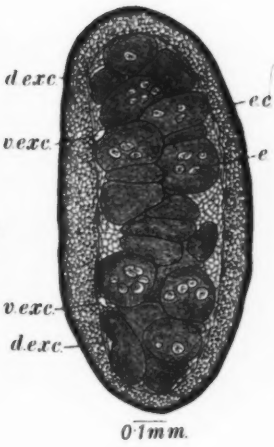


Fig. 7

THE EXCRETORY SYSTEM IN DIGENEA (TREMATODA).

IV. A STUDY OF THE STRUCTURE AND DEVELOPMENT OF THE EXCRETORY SYSTEM IN A CYSTOCERCOUS LARVA, *CERCARIA PEKINENSIS* NOV. SPEC.

By ERNEST CARROLL FAUST.

(*From the Department of Pathology, Peking Union Medical College, Peking, China.*)

(With 6 Text-figures.)

The first record of a cystocercous cercaria was published by Wright (1885). Since that time Braun (1891), Ward (1916), Faust (1918) and Pratt (1919) have contributed to the general morphology of the group. But apart from the collecting reservoir or bladder at the posterior end of the body and the lateral collecting tubules immediately emptying into the bladder the excretory system of these larvae has not been studied. It is the purpose of this paper to lay especial emphasis on this system.

Description of *Cercaria pekinensis* nov. spec.

This larva was obtained from the testis of *Vivipara lapillorum* (Heude), taken from the Grand Canal, outside the East Wall, and from the North Lake, Imperial City, Peking. At first it was not easy to distinguish this species from the large non-bilharzian furcocercous larvae, but a careful study soon made the differences apparent. The movement is characteristically that of the cystocercous larvae, namely, backward instead of forward. The furci are distinctly flapper-like. The posterior portion of the body is partially enveloped by the distal portion of the tail. This tendency of the tail to surround the body is characteristic of the group. In *Cercaria mirabilis*, *C. wrightii*, *C. anchoroides*, and *C. macrostoma* the body is completely enclosed in the anterior portion of the tail, while in *C. stephanocauda* the enveloping tissue is only slightly developed. In this as well as other particulars *Cercaria pekinensis* bears a striking resemblance to *C. brookoveri* (Faust 1918).

C. pekinensis has a body measurement of 0·7 to 0·74 mm. in length by 0·21 to 0·26 mm. in transverse diameter. The tail trunk is 1·5 mm long by 0·21 mm. in diameter while the furci are 0·57 mm. long by 0·16 mm. in transverse measurement. Neither body nor tail has any mammillations or other permanent differentiations of the integument such as characterise most of

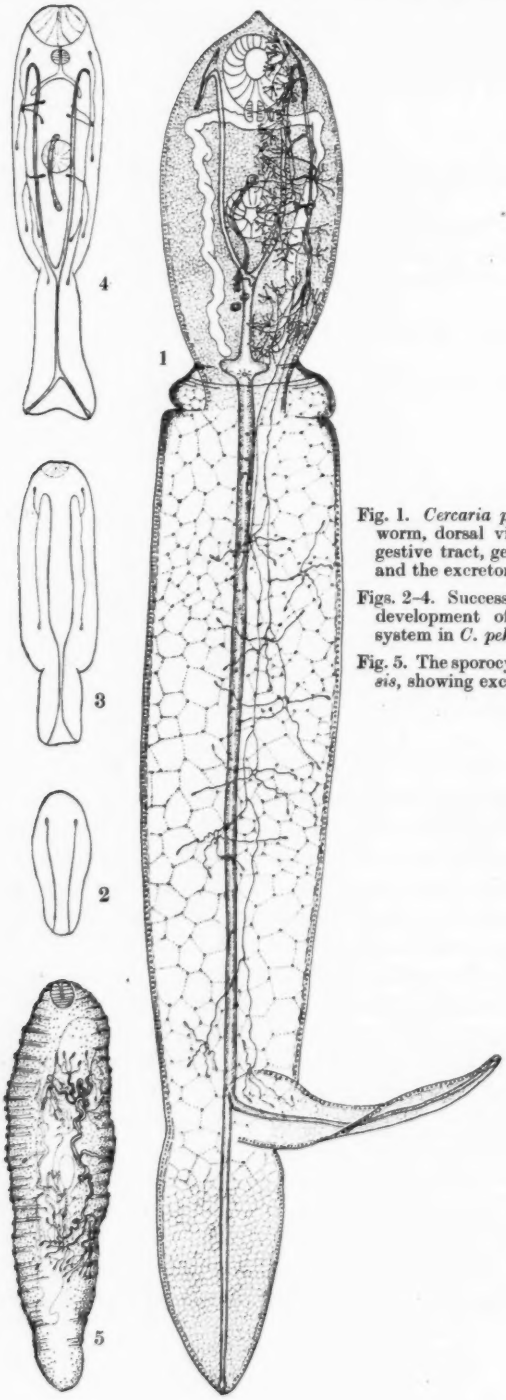


Fig. 1. *Cercaria pekinensis*, entire worm, dorsal view, showing digestive tract, genital cell masses and the excretory system.

Figs. 2–4. Successive stages in the development of the excretory system in *C. pekinensis*.

Fig. 5. The sporocyst of *C. pekinensis*, showing excretory system.

the described species of this group. The oral sucker measures 0·148 by 0·165 mm., while the acetabulum which is situated slightly anterior to the middle of the body measures 0·115 by 0·113 mm. The pharynx has a transverse diameter of 43 μ. Anterior to the acetabulum is the conspicuous genital pore. From the pharynx the coeca first extend laterad and then posteriad, ending just anterior to the excretory bladder. These coeca are long narrow pouches as distinguished from the large thick-walled ones of the closely related species, *C. brookoveri*.

The genital organs are but poorly developed in *C. pekinensis*, even in the most mature individuals. However, the two testes have become differentiated and a lobate body anterior to them is connected by a heavy cord of genital-gland tissue to the genital pore anteriad. In this respect *C. pekinensis* represents a type somewhat more mature than *C. brookoveri*, although it does not approach the degree of maturity of *C. macrostoma*.

Neither cephalic nor cystogenous glands have been seen in the larva. The body parenchyma consists of a close network of tissue. In the tail trunk, however, the parenchyma is extremely loose and vacuolated, with small condensation nuclei only at the interstices. On the other hand the furci have a close network of tissue like the body.

C. pekinensis develops in a sporocyst characterised by an anterior end with pharyngeal sphincter but without a gut. At regular intervals along the body the contracted sporocyst (Fig. 5) has annuli of integument and muscular tissue, which disappear, however, when the animal elongates. In this respect it is strikingly similar to the sporocyst of *C. brookoveri*. The sporocyst with its enclosed larvae has a rapid shuttle-cock movement.

There are two or more generations of sporocysts. Some sporosacs are found to contain nothing but developing sporocysts. Others contain both sporocysts and cercariae. Still others contain cercariae alone. Only a few flukes develop within the parthenita at one time. The tail becomes differentiated from the body soon after the germ-ball stage is passed; but the furci appear late. At the time when the furci first become distinguishable from the tail trunk the oral and ventral suckers are well developed. The pharynx is clearly marked off, but the digestive coeca, while readily observed, only extend a short distance posteriad. At this same stage there is a distinct genital cord extending longitudinally across the face of the acetabulum, suggesting an early sexual maturity in the worm which is not carried out in subsequent development.

No encystment of *C. pekinensis* has been observed nor does the worm readily part with its tail. It seems probable that the fluke passes directly into the subsequent host without a latent period.

The Excretory System.

In studying *C. macrostoma* I noticed numerous flame cells connected with the ultimate capillaries of the excretory system but was unable to work out the pattern on account of the large portion of the body occupied by the mature genital complex. In *C. stephanocauda* I was able to count eight main groups of collecting tubules on each side of the body and in some of these groups I made but sixteen flame cells to each group. *Cercaria pekinensis* has proved very favourable for working out the entire excretory system, not only in the mature cercaria but in typical stages of development and in the sporocyst as well.

In the cercaria, the bladder at the posterior end of the body is compressed oval in shape with a dorsal pore. From the bladder a median trunk extends into the tail, dividing into two trunks somewhat anterior to the bifurcation of that organ. Each of these furcal branches runs to the end of the furcus where it opens to the exterior through a conspicuous pore. These caudal canals receive no lateral branches. They are drainage channels. Coming into the bladder from the anterior side is a single median canal which, when traced forward, divides about two-thirds the distance toward the acetabulum, the two branches of which run toward the oral sucker. They cross over the transverse portion of the corresponding coecum to the side of the oral sucker, in the mid-plane of which they turn back on themselves. As the secondary tubules they are recurrent the entire length of the body (see figures).

In its reversed course the secondary tubule receives eight main (tertiary) branches. The first, third, fifth, and seventh of these provide for excretory drainage of the dorsal portion of the worm, while the alternate ones care for the ventral side. Each of these tertiary tubules, when traced a short distance distad, is found to bifurcate, each fork of which has a definite field of function. This fork in turn receives four tubules at a common centre, and, finally, each of these receives four capillaries, for each of which there is a single solenocyte or flame-cell. Where each group of four tubules unites in a common centre to form the tubule of next higher rank there is a definite polygonal enlargement (see Fig. 6). This is, however, devoid of any concrement.

The anterior four main branches (tertiary tubules) are preacetabular, the fifth is acetabular, the sixth and seventh are postacetabular but within the body, while the eighth provides for drainage of the tail. This latter has an interesting distribution. One ramus cares for the anterior half of the tail trunk (Fig. 1) while the other not only drains the remainder of the tail trunk, but also the flapper of the corresponding side. This elaborate provision for ridding the tail of excretory wastes is apparently unique among cercariae. The furcocercariae have an analogous derivative from the posterior end of the body excretory system but there is no record of such an elaboration of the furcal branch in that group. The condition in the cystocercariae is probably explained by the extraordinary size of the tail common to all described species of the group.

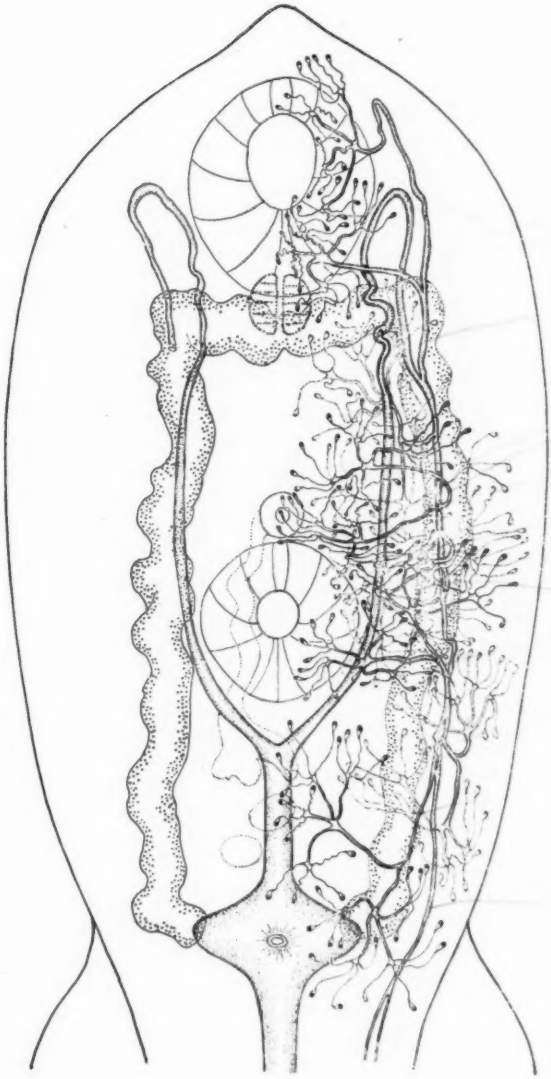


Fig. 6. Body of *Cercaria pekinensis*, enlarged to show pattern of excretory tubules.

Between the third and seventh branches (tertiary tubules) there are several islands of protoplasm (Fig. 6). A study of the younger stages indicates that these were originally lines of separation for the tertiary tubules.

Observation of the excretory system in consecutive periods of development in the fluke makes clear the process of successive differentiation. The young larva just beyond the germ-ball stage (Fig. 2) has a single drainage tubule for each side of the body, opening only at the posterior end and terminating anteriad in a single flame-cell. As the larva develops and the tail region is definitely marked off, the caudal portion of each tubule (Fig. 3) approaches the median line. Meanwhile the flame-cell at the anterior end of the body has divided and the main tubule has become recurrent, so that at the end of this stage there is a single flame-cell in the region of the pharynx and a second flame-cell in a postacetabular position. The next stage (Fig. 4) shows a merging of the two canals in the tail trunk with a separation only in the furcal buds. Each of the two flame-cells in the body has given rise to four cells and the canals have split far backward. This double series of four flame-cells I regard as the fundamental pattern of the excretory system of *Cercaria pekinensis*. Later these tertiary branches separate from one another, providing the eight main branches of the system. By a subsequent bifurcation and two succeeding quadruple splittings the pattern of the developed cercaria is achieved.

In conformity with previous studies in this series (Faust 1919, 1919 *a*, 1919 *b*), the pattern of the system may be expressed as

$$[(2 \times 4 \times 4) + (2 \times 4 \times 4) + (2 \times 4 \times 4) + (2 \times 4 \times 4)] + [(2 \times 4 \times 4) + (2 \times 4 \times 4) + (2 \times 4 \times 4) + (2 \times 4 \times 4)].$$

The primitive pattern may be designated by $\alpha + \beta$, while the fundamental pattern is

$$[\alpha^{i} + \alpha^{ii} + \alpha^{iii} + \alpha^{iv}] + [\beta^{i} + \beta^{ii} + \beta^{iii} + \beta^{iv}].$$

A study of the sporocyst shows that exactly the same pattern obtains. There are two pores, one on each side of the body with a tubule emptying through it. Each main tubule in turn receives an anterior and a posterior branch. Tracing the system further distad each secondary tubule gives place to four tertiary ones, while each tertiary one is the confluence of four capillaries with a flame-cell at each terminus (Fig. 5). It is seen that this pattern is precise, a replica, indeed, of the double fourfold division in each of the quaternary tubules of the cercaria. Taking the entire excretory system on each side of the sporocyst, one sees that it corresponds part by part with a single main (tertiary) tubule of the cercaria. Phylogenetically considered, the system in this species is, therefore, more conservative than is usual in Digenea. Since this same pattern probably obtains in *Cercaria stephanocauda*, it seems possible that the entire group of cystocercous cercariae may have the same fundamental common denominator,

$$[\alpha^{i} + \alpha^{ii} + \alpha^{iii} + \alpha^{iv}] + [\beta^{i} + \beta^{ii} + \beta^{iii} + \beta^{iv}].$$

It likewise seems significant that in the cercaria the pattern has been impressed

twice, for it is represented not only in the main tubules but also in each of the tertiary tubule afferents. Beyond this is the four-fold division into capillaries which apparently represents an addition to the fundamental formula. The more complete formula then is $(\alpha)^2 + (\beta)^2$, while $4\,(\alpha)^2 + 4\,(\beta)^2$ represents the flame-cell total for each side of the cercaria.

DISCUSSION.

The problem presented in this study is a fundamental one. The experimental biologist has shown that the organism obeys within limits certain definite laws, some of which, such as the cleavage of the embryo and the division of sex cells in the earlier stages, are mathematically precise and predetermined. The problem of the excretory system in the trematode is no less a problem in mathematical development. I have previously shown how this obtains in the amphistome group (Faust 1919), in the echinostomes, xiphidiocercariae and furcocercariae of the distome group (Faust 1919 *b*). Cort has confirmed this in his several studies on distome species. Moreover, these larval studies are supported by the monumental work of Looss (1894, 1896) on adult forms. In unpublished studies of holostomes and aspidobothrids I have found the same definite plan of development.

It is of equal significance that the fundamental pattern of excretory tubules of a particular species (representing a group) obtains for the parthenita (sporocyst or redia) of that species. This has previously been demonstrated in *Cercariaeum mutabile* (Cort 1919), in the amphistome, *Cercaria convoluta* (Faust 1919) and in the monostome, *Cercaria spatula* (Faust 1919 *b*). The present paper shows it to obtain in *Cercaria pekinensis*. The pattern is apparently so potentially inherent in the protoplasm that, through the several generations, even profound alterations in other systems and tissues and, indeed, in habits of living, including temperature, tonicity, and H-ion concentration, have practically no effect on this system.

SUMMARY.

1. *Cercaria pekinensis*, a new cystocercous cercaria, is described.

2. The excretory system of the developed cercaria consists of a bladder, a drainage channel through the tail and a pair of anterior collecting tubules, each of which receives eight main (tertiary) branches. Each branch is composed of $2 \times 4 \times 4$ units. The posterior-most branch cares for the drainage of the tail, including the corresponding flapper.

3. A study of representative stages in development shows that the common denominator of the excretory system is $\alpha + \beta$, while the pattern of the developed cercaria is expressed by $4\,(\alpha)^2 + 4\,(\beta)^2$.

4. The fundamental pattern of the cercaria is likewise found in the sporocyst.

5. These data have a significant bearing on the application of mathematics to the problems of growth and development.

REFERENCES.

BRAUN, M. (1891). Die sogenannte "freischwimmende Sporocyste." *Centralbl. f. Bakt. u Parasit.*, Orig. (1), x. 215–219.

COBT, W. W. (1919). A New Cercariaeum from North America. *Journ. Parasitol.* v. 86–91, 1 pl.

FAUST, E. C. (1918). Two New Cystocercous Cercariae from North America. *Ibid.* IV 148–153, 1 pl.

—— (1919, 1919 *a*, 1919 *b*). The Excretory System in Digenea. *Biol. Bull.* XXXVI. 315–344.

—— (1921). Larval Flukes from Georgia. *Trans. Am. Microscop. Soc.* (In press.)

LOOSS, A. (1894). Die Distomen unserer Fische und Frösche. *Bibl. Zool.* XVI. 1–296, 9 pls.

—— (1896). Recherches sur la faune parasitaire de l'Egypte. *Mém. l'Institut Egyptien*, III. 1–252, 16 pls.

PRATT, H. S. (1919). A New Cystocercous Cercaria. *Journ. Parasitol.* v. 128–131.

WARD, H. B. (1916). Notes on Two Free-Living Larval Trematodes from North America. *Ibid.* III. 10–20.

WRIGHT, R. R. (1885). A Free-Swimming Sporocyst. *Amer. Nat.* XIX. 310–311.

A PROTOZOON PATHOGENIC TO MOSQUITO LARVAE.

By W. A. LAMBORN, M.R.C.S., L.R.C.P.,
Medical Entomologist, Federated Malay States.

(*Malaria Bureau, Kuala Lumpur.*)

A BATCH of twenty-nine larvae of *Stegomyia scutellaris* Walker, were collected on 24 July 1920 from an old earthenware pot found in a Malay's garden. It was at once seen, even with the unaided eye, that many, some considerably paler than others, were characterised by a pearly-white opalescence of the tracheal gills at the hinder end of the body.

On microscopic examination it was found that the appearance was due to the presence of a large number of protozoa, which, though in most cases restricted to the gills, had in the paler larvae pervaded the body cavity, and the head even to the interior of the antennae.

The protozoa, which were under ordinary conditions pear-shaped and of considerable size, were usually to be seen careering most actively up and down within the gill as if seeking a point of exit from the larva, but in some cases were packed so tightly as to distend the gill. They were then immobile and spherical. One gill only was usually so distended, sometimes two, and occasionally three and four, but invariably when one gill showed the parasites in large numbers, the others showed them to a less extent.

It was almost impossible to form any correct estimate of the number of organisms present in any one gill. One hundred and fifty-seven were counted in a gill moderately packed with them, and in a gill fully distended it is probable that double that number might be present.

When in such cases a breach of surface of one of the gills was made with a fine needle a large number of the protozoa came out and raced about wildly under the field of the microscope, and it could then be determined that they were provided with flagella.

Evidence of their pathogenicity was afforded by the death in the course of a few days of the entire original brood of larvae. Some two or three, which had shown but few of the parasites, did indeed pupate, and parasites were seen moving slowly within, but none afforded imagoes.

Within a day or two after the death of a larva most of the parasites had left it, though a small number, spherical and probably encysted, remained aggregated together.

It is possible that the softening of the tissues consequent on the death of the larva may be necessary in some instances before the organisms, especially those in the tougher regions of the body, can make their escape. But escape does take place mainly from the larva, while yet alive, either by the rupture, or by the complete separation of the gills; for, on looking through a series of sickly larvae, it was noticed that often one, sometimes two and rarely all the gills were absent; and by placing larvae having the full complement of gills but showing infection, each in small separate bowls, the facts as to rupture and separation of the gills were verified.

Larvae did not seem to be greatly affected by the loss of one gill, and occasionally specimens which had lost all four were observed. None were ever known to pupate, though one larva without any gills managed almost completely to detach most of its skin in attempted pupation, then dying with the skin still enveloping its last two or three segments.

On the death of the last larva a fresh batch of fifty half-grown *Stegomyia* larvae were put into the water; most of these pupated and a few died, but showed no evidence of the presence of the protozoa. Thereafter until October 16, almost three months later, batches of the larvae were from time to time put in, keeping the number constantly between thirty and fifty, a little of the fluid in which they had occurred being added to make up for the evaporation of the original water.

During all these weeks there was a constant succession of pupations or deaths among the larvae and it was not until this date that the protozoa again showed evidences of their presence. It is perhaps noteworthy that during the last fortnight the bowl, which had been kept in a well-lighted situation, had been removed to a dark corner of the laboratory.

On October 16 three of the larvae, floating at the surface, were seen each with one milky-looking gill in which, on microscopic examination, the protozoa were found to be swarming, the other three gills being affected to a less degree.

An examination was then made of every larva and out of twenty-seven, thirteen were apparently healthy, while fourteen, some lacking one or more gills, showed the presence of the parasites to a greater or less extent.

The appearances presented by each of the affected larvae, most of which were approaching maturity, were as follows, the specimens being numbered 1–14:

(1) Alive; one gill showing extreme distension with organisms; one gill about half-full; other gills showing one organism each: very moderate body infestation, a few only here and there.—(2) and (3) As specimen (1).—(4) Dead: had lost one gill. In one of the remaining gills were about twenty of the parasites, spherical, immobile and apparently attached to each other, for they moved *en masse* when some of the living organisms, of which there were about ten, butted against them. In each of the other two gills were about an equal number of resting and actively moving organisms, about twenty in all. These teemed in the body.—(5) Alive; had lost two gills. The remaining gills showed

a moderate infestation with organisms all of which were active. There were a few in the body.—(6) Alive; all four gills were present, one half-full of parasites; one containing about twenty, the others about ten each; all very active: parasites present in great abundance in body especially posteriorly, all active.—(7) Alive; but moribund; lying flat on water: one gill only present. There were no organisms in it and there was no body infestation.—(8) Alive: had three gills only; no infestation either of these or of body.—(9) Alive; no gills at all: slight infestation of body: all organisms very active.—(10) Alive: only two gills were present; one full of granular material, one showing at base about seventeen organisms at rest; a few scattered throughout body.—(11) Alive; all gills were present and in each of three were two organisms, the fourth showing none: in the body were a few scattered organisms; not more than about ten in all.—(12) Alive; only one gill was present, containing about thirteen active organisms: none to be seen in body.—(13) Alive; half-grown, had two gills only; one full of organisms, one half-full; all active; showed a few, perhaps ten in all, in body.—(14) Alive; had one gill only; but marked infestation of the body, especially posteriorly.

ON A NEW CILIATE: *LAMBORNELLA STEGOMYIAE* N.G., N.SP., PARASITIC IN THE BODY-CAVITY OF THE LARVAE OF *STEGOMYIA SCUTELLARIS* WALKER (DIPTERA, NEMATOCERA, CULICIDAE).

By D. KEILIN, Sc.D.,

Beit Memorial Research Fellow.

(*From the Quick Laboratory, University of Cambridge.*)

(With 6 Text-figures.)

CONTENTS.

		PAGE
I.	Introduction	216
II.	Description of *Lambornella stegomyiae*, n.g., n.sp.	217
III.	Reproduction	220
IV.	Encysted stage	220
V.	Mode of Infection	222
VI.	Diagnosis of *Lambornella stegomyiae*	223
VII.	Appendix: (*a*) The Ciliate Parasites of Insects	223
	(*b*) The Ciliate Parasites of Arthropods in general	223
VIII.	References	224

I. Introduction.

The material which forms the subject of this communication was derived from Dr W. A. Lamborn's collection, and was placed at my disposal through the kindness of Dr Guy A. K. Marshall, Director of the Imperial Bureau of Entomology, London. It comprised eight larvae of *Stegomyia scutellaris* Walker, which had been preserved in formaldehyde solution (10 per cent.), and the specimens were mounted individually on microscope slides in the same medium.

Examined in transmitted light, the preparations showed the body-cavities of the larvae to contain large numbers of the parasites, a feature which had already been described by Lamborn (1921).

From the nature of the preparations, it may be imagined that it was a matter of no small difficulty to carry out the detailed examination of the cellular structure of the organisms. It was, of course, necessary to remove specimens from the body of the host for the purpose of cytological study. The procedure which I adopted was as follows: the larvae were first examined *in toto*, and sketches were made in order to show the general distribution of the parasites in the larval body.

Some of the larvae were then divided into three pieces, and transferred from the formalin medium in which they were mounted into distilled water, in which they were allowed to remain for about an hour. They were then stained in a strong solution of haemalum, differentiated in alcohol (30 per cent.) to which a little hydrochloric acid had been added, and washed for a few minutes in tap-water. The pieces were then passed slowly through the graded alcohols, cleared in clove oil followed by xylol, and then into very liquid Canada balsam. Some of the pieces were mounted whole, but others were teased up in a drop of balsam, using a high magnification of the binocular microscope and very fine needles, with the object of liberating the stained parasites from the body of the host. When the parasites were well scattered in the balsam, a cover-glass, which had been momentarily immersed in xylol, was dropped on.

Other portions of the larvae were embedded in paraffin and sections cut, but the latter proved to be less instructive than the teased preparations.

Of the eight larvae examined, two proved to be free from parasites; they had been included in the lot on account of their mutilated condition, both having been found dead, with three gills missing, a condition which it was thought by Lamborn might be attributed to a parasitic infection. Five of the larvae showed the presence of the Ciliate parasite, but the sixth was the host of a fungal parasite with which I am dealing in another paper.

II. *LAMBORNELLA STEGOMYIAE*, n.gen., n.sp.

The ciliate about to be described is a new form, and I have named it in honour of its discoverer—Dr W. A. Lamborn[1].

The parasite is found distributed throughout the body-cavity of the host, from the head to the anal segment, and even penetrates to the respiratory siphon and the gills. Fig. 1 shows the appearance of the posterior extremity of a parasitised larva as seen by transmitted light. Sections of the same larva show no obvious damage to the internal organs, with the exception of the fat-body which, in places, has disappeared completely, while the parts which remain are reduced to a few cells devoid of fat-droplets.

The condition of each of the five parasitised larvae was as follows:

Larva No. 1. One of the four gills missing; body filled with parasites, as represented in Fig. 1.

Larva No. 2. Parasites scattered throughout the body-cavity; all four gills present, one of which is tightly packed with parasites, another moderately invaded, and the other two with very few ciliates; parasites very numerous in the respiratory siphon, surrounding the tracheal trunks.

Larva No. 3. Ciliates numerous in the posterior segments, scattered in the head and the remaining segments; two of the gills are missing, one of the remaining gills contains numerous parasites in its proximal portion only, the

[1] The name *Lambornia* is preoccupied for an Insect (Lepidoptera, Geometridae; Prout, *Genera Insectorum*, 1912, p. 235).

other is free from infection. As will be seen later, this larva yielded *Lambornella* cysts.

Larva No. 4. Contains few parasites; labelled by Lamborn "early infestation with protozoal parasites."

Larva No. 5. Found dead, with only one gill remaining; few parasites scattered throughout the body-cavity.

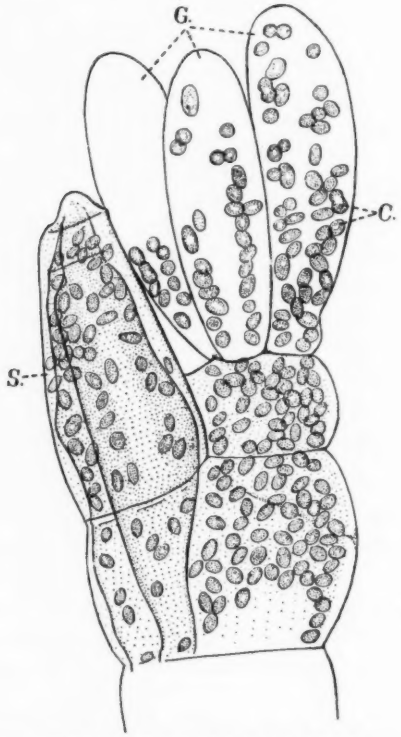


Fig. 1. Larva of *Stegomyia scutellaris* Walker, posterior end of the body, siphon and gills showing by transparency a very great number of parasitic ciliates (*Lambornella stegomyiae*). The contours of the larva are represented schematically, all the accessory organs and sensory hairs being omitted.

In the living state, the body of *Lambornella stegomyiae* is described by Lamborn (1921, p. 213) as being pear-shaped; in a few cases specimens fixed in formalin still retain this shape, but, more often they are elongately oval, ranging in length from 50μ to 70μ, with a maximum diameter of 20μ to 30μ. The body surface is uniformly covered with cilia, densely disposed, and arranged in longitudinal parallel rows. The ectoplasmic layer is not clearly defined; the endoplasm is very granular. The macronucleus (Figs. 2 and 3, *M.*) is spherical, from 10μ to 13μ in diameter, stains deeply and uniformly in

haemalum or haematoxylin, showing neither granules nor vacuoles. The micronucleus (Figs. 2 and 3, *m.*) appears as a small chromatic granule, 2μ to 3μ in diameter, and usually lies in a small peripheral depression of the macronucleus. Its apparent position depends on the orientation of the body of the parasite, and varies from a marginal to a central position, but careful examination shows that its position is superficial on the macronucleus, sunk into a shallow depression or notch. In a few cases in which I have observed the

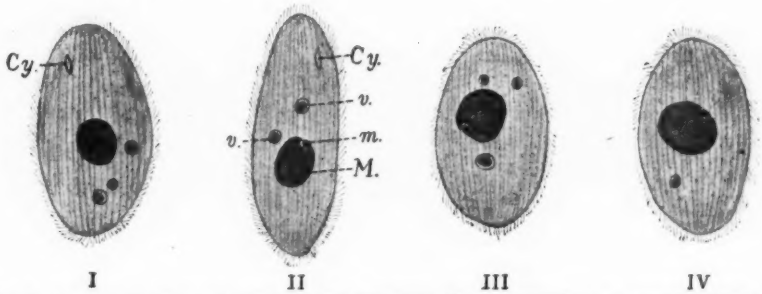


Fig. 2. *Lambornella stegomyiae*, showing the cytostome, *Cy.*; micronucleus, *m.*; macronucleus, *M.*

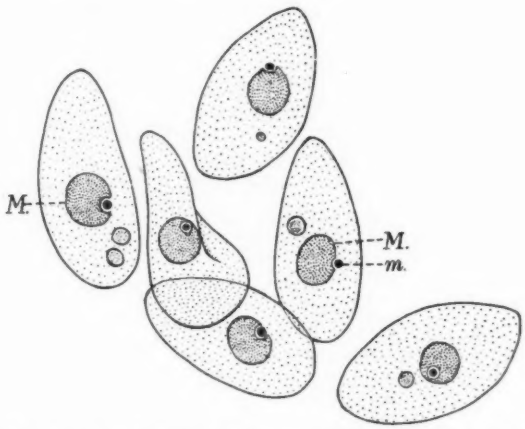


Fig. 3. *Lambornella stegomyiae*. A group of six specimens of this ciliate as seen in one field of the microscope. Letters as in Fig. 2.

micronucleus widely separated from the macronucleus, the latter invariably showed an empty depression, from which the micronucleus had doubtless been displaced accidentally. In addition to the two nuclei, the protoplasm contains one to four vacuoles which stain uniformly with basic dyes. Notwithstanding its purely parasitic mode of life, *Lambornella* possesses a small cytostome. The cytostome is difficult to see in fixed and stained specimens, and I thought at first that I was dealing with a ciliate belonging to the group of the Astoma,

which are all known to be parasites of animals. Finally, however, by careful search under a high magnification, I have succeeded in demonstrating its presence. It appears as a small, lozenge-shaped, refractile spot (Fig. 2, I and II, *Cy.*), interrupting the longitudinal striations of the body surface. It is ventral in position and forms a small groove, situated at a distance of 10μ from the anterior end of the body, and measuring 5 to 6μ in length and 2 to 3μ in width. The cytostome is most easily seen when viewed in profile.

III. Reproduction.

Reproduction of *Lambornella stegomyiae* takes place by simple, transverse fission. The micronucleus first divides mitotically, and when the two daughter micronuclei have appeared, the macronucleus undergoes an amitotic division, accompanied by a constriction of the body and fission of the protoplasm (Fig. 4, *A* and *B*). I have not seen any evidence of conjugation.

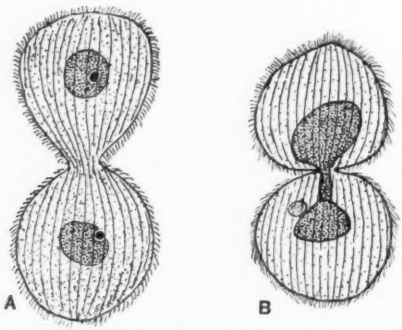


Fig. 4. *Lambornella stegomyiae.* Two dividing specimens of the ciliate.

IV. Encysted Stage.

Among the hundreds of parasites seen within the bodies of the *Stegomyia* larvae, no cysts were present; all the ciliates were in the active or trophic phase. One of the larvae only (*see* p. 218, No. 4) showed the cystic form of *Lambornella*. The external surface of the cuticle of this larva was studded with small, transparent, hemispherical vesicles, each of which enclosed a contracted mass of protoplasm (Fig. 5). These I first thought to be epizooic organisms such as are commonly found upon the bodies of fresh-water Arthropods. In stained preparations, however, the protoplasmic mass was seen to contain a nucleus so closely resembling in its structure that of *Lambornella* as described above, that no doubt remains in my mind: these vesicles are unquestionably the cysts of the ciliate.

The cysts measure from 30μ to 40μ in diameter and 20μ in height (Fig. 6). The protoplasmic mass (22μ to 32μ in diameter) contains a contracted macronucleus (8μ to 10μ in diameter) with a peripheral depression in which the

small micronucleus is borne. In a few individuals, I was still able to perceive the faint superficial striation of the protoplasm and one or two basophile vacuoles. Several cysts showed a clear vacuole ranging from 3μ to 8μ in diameter. The wall of the cyst is very thin and does not show any definite structure.

From the foregoing, it is evident that the parasite passes through a period of rapid multiplication within the body of the *Stegomyia* larva, after which

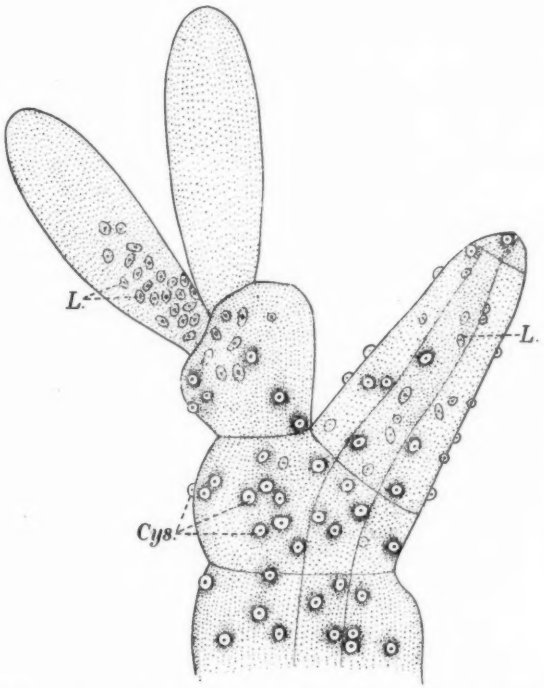


Fig. 5. Larva of *Stegomyia scutellaris* Walker, posterior end of the body showing by transparency several specimens of active stages of *Lambornella stegomyiae*, *L.* and many cysts of this ciliate, *Cys.*, fixed upon the cuticle of the larva.

it leaves its host and fixes itself on the external surface of the cuticle (and, possibly, to any adjacent solid object), and there becomes encysted. The parasites probably leave the body of the host through artificial ruptures of the body-wall, and as the gills are the most fragile of the appendages of the larva, it appears to be likely that the parasites reach the exterior through the openings which are left after the gills have broken away (see Lamborn, p. 214). In connection with this, it is interesting to note that larva No. 4, which was covered with cysts of *Lambornella*, had lost two of its gills.

The formation of external cysts attached to the body of the host has been observed in other ciliates parasitic in the body-cavity of Arthropods. According to Balbiani (1885), *Collinia circulans* (Balbiani) Cépède 1910, which lives as a parasite in the body-cavity of *Asellus aquaticus*, after passing through a phase of active multiplication in the body-cavity of the host, escapes through ruptures in the antennae and attaches itself to the external cuticle of the host, or to the surrounding filaments of the alga *Cladophora*, where it becomes encysted. A similar mode of encystment has been observed by Cépède and

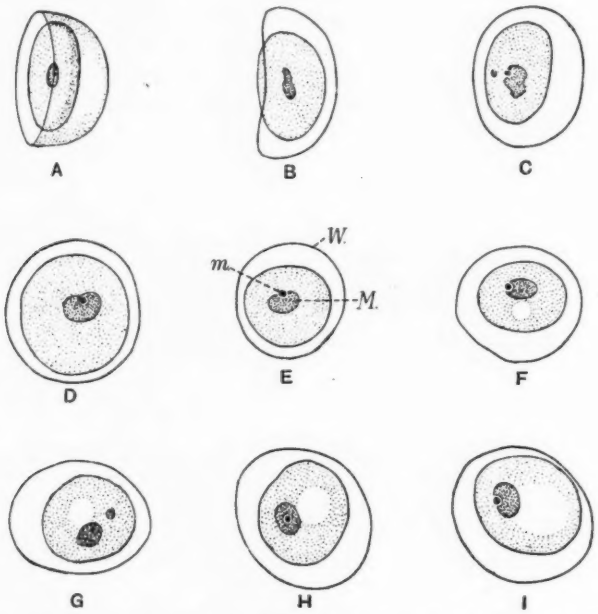


Fig. 6. *Lambornella stegomyiae*. Different forms of the cysts of this ciliate; *m.* micronucleus; *M.* macronucleus; *W.* wall of the cyst.

Giard (1910) in *Perezella pelagica* Cépède, a ciliate parasite found in the body-cavity of marine Copepods.

V. Mode of Infection.

In his note (p. 214), Lamborn records that he has succeeded in infecting healthy *Stegomyia* larvae by keeping them with infected living and dead specimens of the larva. Infection is probably acquired through the mouth in the case of *Lambornella*, but it should be mentioned that Balbiani failed to infect healthy *Asellus* by feeding them upon the cysts of *Collinia*. It is possible that the failure was due to the fact that the cysts were unripe.

It is highly probable that parasitic ciliates such as *Collinia* and *Lambornella* undergo a process of multiplication within the cyst-wall, comparable to that which is observed to occur in *Ichthyophthirius*, a parasite of fresh-water fishes, and that only those cysts filled with young ciliates, which are capable of penetrating the walls of the alimentary canal of the host, are infective when ingested by the host.

That the cysts of *Lambornella* must pass through some developmental phase before they become infective, is supported by Lamborn's experience, in that he obtained successful results in his infection experiments not less than three months after the death of the larvae which he used as a source of infection.

VI. DIAGNOSIS OF *LAMBORNELLA*, n.gen.

Diagnosis: a holotrichous parasitic ciliate. *Body* elongate oval, length 50μ to 70μ, maximum width 20μ to 30μ. *Ciliation* dense. *Cytostome* small lozenge-shaped. *Macronucleus* spherical, 10μ to 12μ in diameter; *micronucleus* 2μ to 3μ in diameter, lying in a peripheral depression of the macronucleus. *Protoplasm* granular, with few basophil vacuoles. Multiplication takes place by simple transverse fission into two equal parts. *Cysts* hemispherical, 30μ to 40μ in diameter, 20μ high, transparent and structureless, attached to the host's cuticle.

One species known:

Lambornella stegomyiae, n.sp.

Diagnosis: as that of the genus.

Habitat: the body-cavity and gills of *Stegomyia scutellaris* Walker (Diptera, Nematocera, Culicidae).

Description based on material collected by Dr W. A. Lamborn at Kuala Lumpur, Federated Malay States.

VII. APPENDIX.

(*a*) *The Ciliate Parasites of Insects.*

The only example of a ciliate, parasitic in the alimentary canal of insects, which has been recorded hitherto, is *Nyctotherus*, the hosts of which are adults of *Blatta*, *Gryllotalpa*, and *Hydrophilus*, and the larva of *Oryctes* (*see* Bütschli, 1899, p. 1721).

To the best of my knowledge, *Lambornella stegomyiae is the first and only instance of a ciliate parasitic in the body-cavity of an Insect.*

(*b*) *The Ciliate Parasites of Arthropods in general.*

It is a matter of some surprise to find how few ciliate parasites of Arthropods are known: I append the following list:

1. *Anophrys maggi* Cattaneo, parasitic in the blood of *Carcinus maenas*. The species is very insufficiently described.

2. *Collinia branchiarum* (Stein, 1852) Cépède 1910, parasitic in the gills of *Gammarus pulex*.
3. *Collinia neoniphargi* Cépède 1910, parasitic in the circulatory system of *Neoniphargus moniezi*.
4. *Collinia circulans* (Balbiani, 1885) Cépède 1910, parasitic in the body-cavity of *Asellus aquaticus*.
5. *Perezella pelagica* Cépède 1910, parasitic in the body-cavity of Copepoda (Calanidae), *Clausia elongata* Boeck., *Acartia clausi* Giesbrecht, and *Paracalanus parvus* Claus.
6. *Uronema rabaudi* Cépède 1910, parasitic in the body-cavity of *Clausia elongata* and *Acartia clausi*, also in the dead bodies of these forms.
7. *Nyctotherus* sp.? in the hind-gut of *Blatta*, *Gryllotalpa*, and *Hydrophilus* adults, and the larva of *Oryctes*; also in *Iulus* (Myriapoda) (*see* Bütschli, 1899, p. 1721).
8. *Lambornella stegomyiae*, n.gen., n.sp., parasitic in the body-cavity of the larva of *Stegomyia scutellaris* Walker.

VIII. References.

Balbiani, E. G. (1885). Sur un Infusoire Cilié parasite du sang de l'Aselle aquatique (*Anoplophrya circulans*). *Recueil Zoologique Suisse*, II. 276–303, Pl. XI.

Cépède, C. (1910). Recherches sur les Infusoires Astomes, Anatomie, Biologie, Ethologie parasitaire, Systématique. *Arch. Zool. Expér. et Génér.* 5e série, III. 341–609, Pls IX—XVII.

Howard, L. O, Dyar, H. G. and Knab, F. (1912). *The Mosquitoes of North and Central America and the West Indies*. Washington, Carnegie Institution, I. 157–161.

Lamborn, W. A. (1921). A Protozoan pathogenic to Mosquito Larvae. *Parasitology*, XIII. 213–215.

ON A NEW TYPE OF FUNGUS: *COELOMOMYCES STEGOMYIAE* N.G., N.SP., PARASITIC IN THE BODY-CAVITY OF THE LARVA OF *STEGOMYIA SCUTELLARIS* WALKER (DIPTERA, NEMATOCERA, CULICIDAE).

By D. KEILIN, Sc.D.,

Beit Memorial Research Fellow.

(*From the Quick Laboratory, University of Cambridge.*)

(With 7 Text-figures.)

CONTENTS.

		PAGE
I.	Localisation of the parasite in the body of *Stegomyia* larva	225
II.	Mycelium of the parasite	226
III.	Development and structure of sporangia	228
IV.	Development and structure of spores	230
V.	Probable mode of infection	231
VI.	Systematic position of *Coelomomyces stegomyiae*	232
VII.	Parasites of mosquito larvae	232
VIII.	References	234

I. LOCALISATION OF THE PARASITE IN THE BODY OF *STEGOMYIA* LARVA.

Among six parasitised larvae of *Stegomyia scutellaris* Walker, collected by Dr W. A. Lamborn in the Federated Malay States, five contained a ciliate which I have described (*Parasitology*, XIII. p. 216) under the name of *Lambornella stegomyiae* n.g., n.sp., while the remaining larva harboured a new parasitic fungus which forms the subject of this communication. The larva had been fixed and was preserved in formaldehyde solution (10 per cent.) and was labelled: "Larva of *Stegomyia scutellaris* Walker. Infestation with protozoal parasite and a luxuriant growth of Vorticella. Kajang," by Dr Lamborn. When the larva was examined under a low power the surface of its body was found to be extensively covered with tufts of *Vorticella*, whilst its interior harboured an enormous number of parasites which in the gills and the posterior segments were packed in solid masses completely filling these parts (Fig. 1). As seen by transparency the parasites are oval in shape, 37·5 to 57 μ long and 20 to 30 μ in diameter, surrounded by a more or less thick yellowish wall. In their external appearance, size, and position in the host, they are so surprisingly similar to *Lambornella* that it was at first quite natural to take them for the resting stages or cysts of this ciliate. It is sufficient to compare Fig. 1 of this

paper with Fig. 1 (p. 218) of the paper on *Lambornella* to appreciate this misleading resemblance. The detailed study of the structure of this new parasite, however, showed that we were dealing not with the cysts of *Lambornella*, but with a totally different organism belonging to the Fungi.

For the study of this new parasite which I propose to name *Coelomomyces stegomyiae* n.g., n.sp., the best results were obtained (*a*) by teasing up pieces of the larva in lactophenol, either pure or after the addition of a drop of cotton-blue, and (*b*) by studying sections of the larva stained in glychaemalum or iron haematoxylin and eosin.

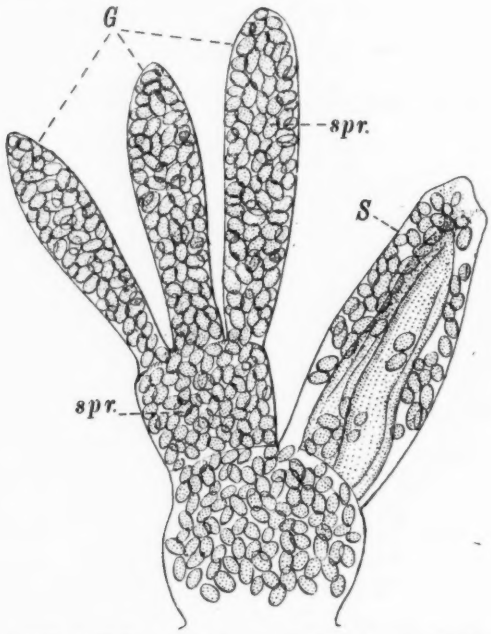


Fig. 1. *Stegomyia scutellaris* Walker. Posterior extremity of larva with three gills, *G*; and the respiratory siphon, *S*; packed with sporangia—*spr.* of *Coelomomyces stegomyiae*.

The sections show that, although the larva is very heavily infected and lacking one gill, its internal organs are apparently healthy, but for the fat body which has completely disappeared (Fig. 2).

II. MYCELIUM OF THE PARASITE.

Portions of the parasitised larva, examined in the manner described, showed not only the oval bodies previously referred to, but also fragments of true mycelium. There is little mycelium in the body-cavity, only a few branches being seen in sections, but the mycelium is well-developed immediately around the viscera, especially the midgut (Fig. 4) and the five anterior intestinal coeca,

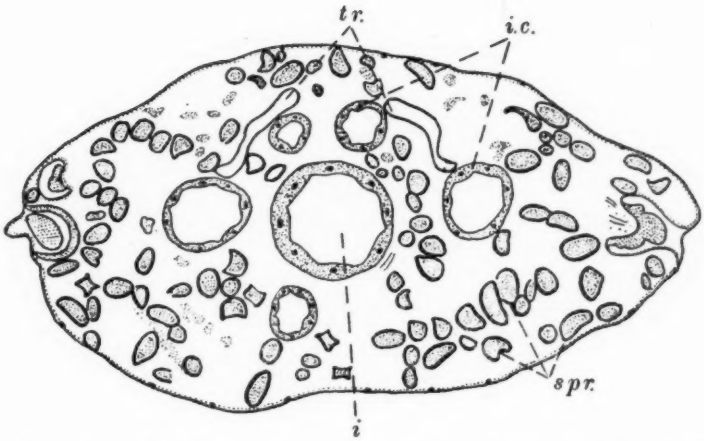


Fig. 2. *Stegomyia scutellaris* Walker. Transverse section of an abdominal segment of the larva showing the sporangia, *spr.*, of *Coelomomyces*; *i*, midgut; *i.c.*, intestinal coeca; *tr.*, tracheal trunks.

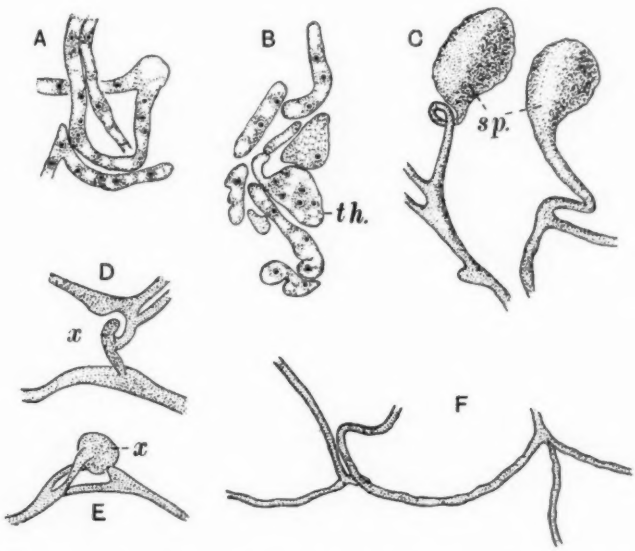


Fig. 3. *Coelomomyces stegomyiae*. *A* and *B*, sections of the mycelium stained in glychaemalum: *th.*, terminal thickenings which become transformed into sporangia; *C*, fragments of mycelium with terminal swellings in a more advanced stage; *D* and *E*, fragments of mycelium showing transverse branches with diverticula or spherical thickenings, *x*; *F*, the ordinary form of mycelium.

forming two to three concentric layers, so closely attached to the organs, that in some places it is difficult to separate the tissues of the host from the surrounding mycelium. The latter is also well-developed beneath the hypoderm of the host, where it is covered with the pigmented remains of the peripheral cells of the fat body (Fig. 5 *A*). Examined in lactophenol, the fragments of mycelium show numerous ramifications, the branches varying from 2 to 6 μ in thickness; while some fragments are more regularly ramified and about 3 μ thick (Fig. 3 *F*), the others are composed of entangled branches with irregular thickenings which seem to be formed by a confluence of two or more branches; in such places the mycelium attains a width of 5 to 6 μ (Fig. 4 *my*).

The main branches of the mycelium are often connected by short transverse branches which, in places, show a small diverticulum or a spherical thickening (Fig. 3 *D* and *E*, *x*). The mycelium, examined *in toto* in lactophenol-cotton blue, or in stained sections, did not show any transverse walls and is therefore unicellular. Its nuclei are very chromatic, crowded in places, and scattered elsewhere. Each nucleus is surrounded by a dense layer of protoplasm, which in the other parts of the mycelium is filled with vacuoles of various sizes and shapes (Fig. 3 *A* and *B*).

III. DEVELOPMENT AND STRUCTURE OF SPORANGIA.

The majority of the branches show terminal thickenings of various sizes; in some branches they are hardly noticeable, while in the others they form a pronounced terminal swelling 30 to 35 μ long and 20 to 22 μ thick (Fig. 3 *C*, *sp.*). The contours of these terminal dilatations are somewhat irregular and their surface often shows a series of very fine transverse wrinkles. These thickened portions are very difficult to stain *in toto*, but in sections they show the same structure as that of the ordinary mycelium, with the difference that they contain a larger number of nuclei, and the protoplasm is more vacuolated (Fig. 5 *A*). At a later stage of development the terminal swellings become separated from the mycelium and are found free within the insect's body-cavity, their length varying from 32 to 65 μ. At first they possess a structure identical with that of the terminal swelling of the mycelium, but they appear to increase in size after becoming free in the host's perivisceral fluid. The number of their nuclei varies with their size.

At a later stage these bodies become more regularly oval; the peripheral layer of protoplasm grows denser (Fig. 5 *C*) and the external wall thickens (Fig. 5 *D*, *w.*). The nuclei now increase in number, undergoing several divisions, and the protoplasm becomes more dense and basophile. The wall thickens progressively and assumes a complicated structure, ending in the development of sporangia, the form of the parasite that is most frequently found in the perivisceral cavity of the host. The sporangia constitute the main mass of parasites which fills the posterior segments of the host (see Fig. 1). When removed from the larva the sporangia appear as oval bodies measuring 37·5 to 57 μ in length and 20 to 30 μ in width, flattened on one side and convex on

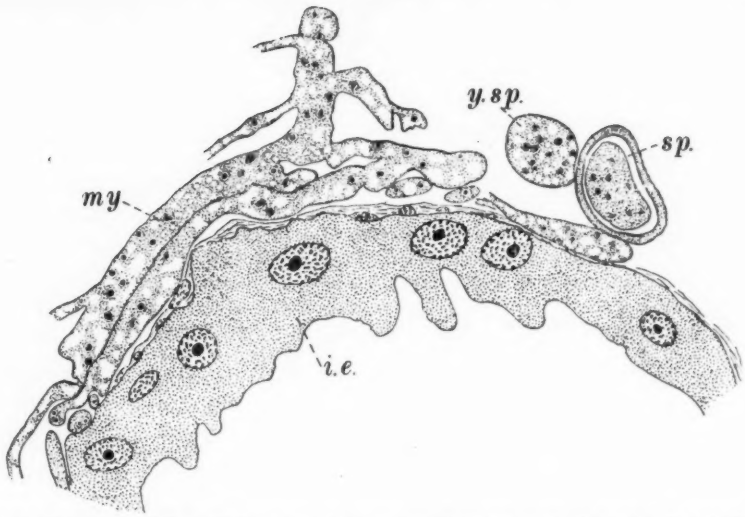


Fig. 4. *Stegomyia scutellaris* Walker larva; transverse section of the midgut showing the intestinal epithelium, *i.e.*, surrounded by a mycelium, *my*, of *Coelomomyces*; *y.sp.*, young sporangium; *sp.*, sporangium with thickened wall.

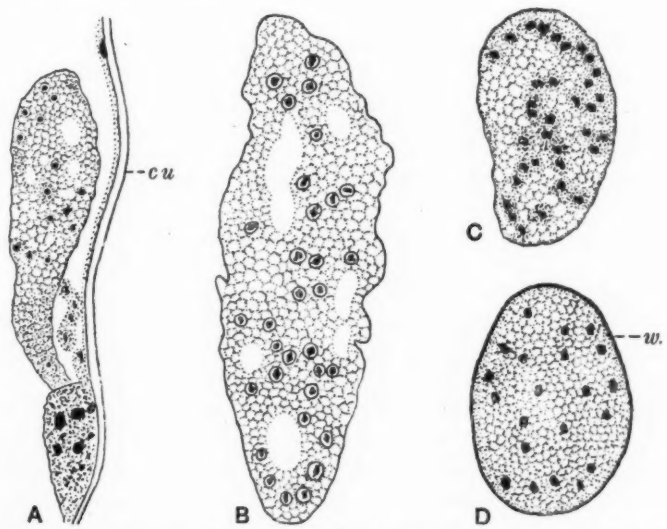


Fig. 5. *Coelomomyces stegomyiae*. *A*, section of a terminal thickening of mycelium which develops into a sporangium; *cu.*, cuticle of the larva; *B*, section of a terminal thickened portion of mycelium at a more advanced stage when it is detached from the mycelium and is free in the perivisceral fluid of the host; *C*, section of an early stage in development of a sporangium showing the condensation of the peripheral protoplasm; *D*, young sporangium showing the beginning of the thickening of the wall, *w*.

the other (Fig. 6 *A*). The wall of the sporangium when highly magnified shows a very fine granular structure and many small clear lenticular spots (Fig. 6 *B*, *a.*); the convex side shows a very fine line running from pole to pole.

Sections of the sporangium show that the wall consists of two distinct layers: (1) an internal, thin, structureless layer, 0·7 μ thick (Fig. 7 *A*, *en.*), and (2) an external layer (Fig. 7 *A*, *ex.*) 1·7 to 2 μ thick, of a yellowish colour, and showing numerous apertures (*a.*) already referred to as clear spots on the surface of the sporangium. By pressing a sporangium in a drop of water between the slide and a coverglass a number of small oily drops are seen to escape from the apertures of the external wall.

IV. DEVELOPMENT AND STRUCTURE OF SPORES.

The young sporangium is filled with very dense and basophile protoplasm, containing a great number of small nuclei (Fig. 7 *B*). As the sporangium

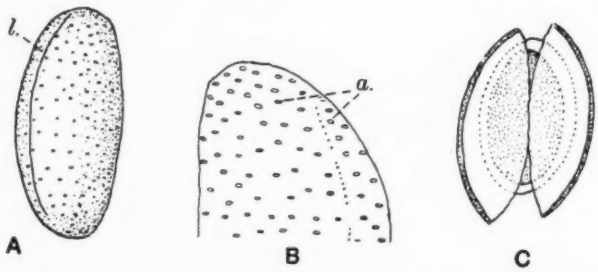


Fig. 6. *Coelomomyces stegomyiae*. *A*, a completely formed sporangium showing the clear line, *l.*, upon its convex side; *B*, sporangium showing the distribution of small apertures, *a.*, upon its external wall; *C*, breaking up of a sporangium under pressure.

develops the protoplasm divides into many portions, each of which surrounds a nucleus (Fig. 7 *C*) and gives rise to as many small spherical cells as there were nuclei (Fig. 7 *D*). These cells, 3 μ in diameter, become elongated, with a more or less central nucleus (Fig. 7 *E* and *F*). The sporangia filled with these lozenge-shaped spores, 5 μ long and 1 μ in diameter, were not very numerous, and they represent the last stage of the parasite which I could find in the host. Unfortunately the available material was insufficient for a more detailed study of these spores. Their shape, however, and the fact that they probably represent the final infective stage of a parasite of an aquatic host suggest that these spores are provided with one or two flagella and are the actual flagellispores or zoospores which are so characteristic of some groups of lower fungi; this supposition could be readily demonstrated by an examination of living material.

V. PROBABLE MODE OF INFECTION.

The escape of these spores probably results from the rupture of the sporangium along the clearly marked line of cleavage that runs along its convex surface. I was able to observe such rupture of the sporangium by compressing

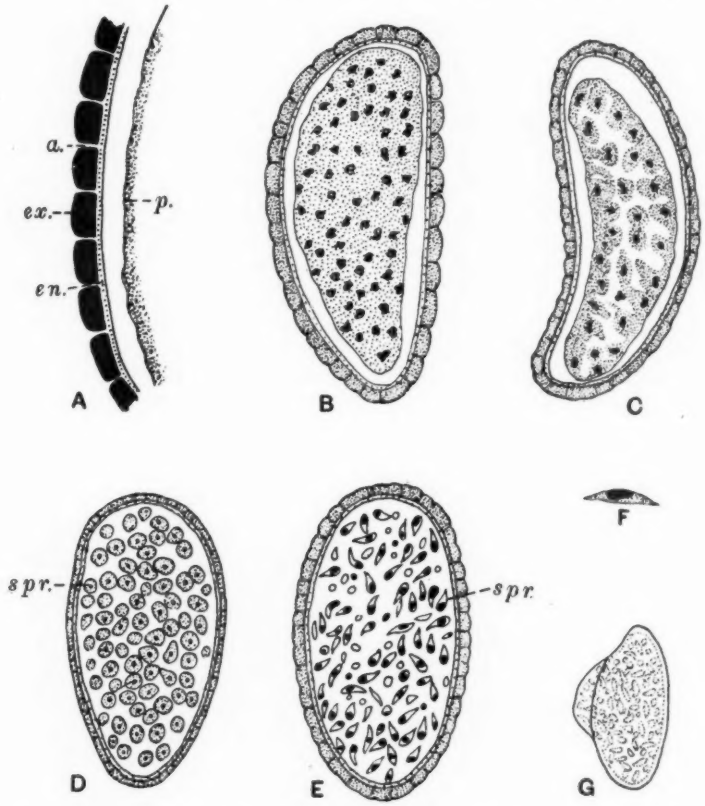


Fig. 7. *Coelomomyces stegomyiae.* *A*, section of the wall of sporangium: *a.*, apertures; *en.*, internal structureless membrane; *ex.*, external thick perforated membrane; *p.*, central protoplasmic content; *B*, section of an unripe sporangium; *C*, section of sporangium showing the beginning of the breaking up of its content into separate cells; *D*, sporangium with a thin wall and content divided into a great number of spherical cells, *spr.*; *E*, section of a sporangium showing the completely formed spores, *spr.*; *F*, spore (or probably a zoospore) from a sporangium similar to that represented in Fig. *E*; *G*, a small sporangium with spores, seen *in toto* the external layer having ruptured and the internal thin membrane bulging out from the slit.

it between the slide and a coverglass. When the external wall of the sporangium is ruptured, the thin internal wall bulges out and by its rupture allows the spores to escape. It is possible that the protruded internal wall of the

sporangium is capable of perforating the chitin of its host, whereby, as in some parasitic Olpidiaceae, the spores are conducted out of the host into the water.

It should be mentioned that two kinds of sporangia were found by me in the body-cavity of the *Stegomyia* larva: (1) sporangia with a very thick wall (Fig. 7 *A*, *B*, *C* and *E*), and (2) sporangia with a thin wall (Fig. 7 *D* and *G*), and this independent of the stage and the content of sporangia. It is possible that the latter serve for immediate reproduction, while the former represent a resistant or resting form of the parasite.

VI. SYSTEMATIC POSITION.

It is difficult to allocate this organism to its systematic position. Since the mycelium of *Coelomomyces* is devoid of transverse walls (*i.e.* it is unicellular), it would appear to belong to the Phycomycetes and it undoubtedly shows some resemblance to the Chytridineae[1]. In this group, *Physoderma* (Cladochytriaceae) and *Catenaria* (Hyphochytriaceae) have a more or less similar development of the sporangia within the tissue or body-cavity of the host and they possess a more developed mycelium than other Chytridineae. The structure of the sporangia of *Coelomomyces* differs, however, in many respects from that of the Chytridineae and its mycelium appears to be better developed than is the case in any known representative of this group of Fungi. The systematic position of *Coelomomyces* cannot be finally established until more abundant and living material is available for a detailed study of its structure. It is still necessary to determine (1) the structural character of the mycelium during the early stages of infection, before it is transformed more or less completely into sporangia; (2) whether conjugation occurs, and at what stage of development of the parasite; (3) the structure of the spores; (4) the mode of their liberation from the body of the host and, finally, (5) the mode of infection of a new host and the formation of the first mycelium.

Several other matters of general interest still await solution, and, first of all, it is desirable to know if the parasite is confined to but one species of *Stegomyia* or if it infects other species of Culicidae or perhaps other Arthropods.

Acknowledgement. My thanks are due to Mr F. T. Brooks, M.A., University Lecturer in Botany, Cambridge, for his helpful suggestions in connection with this study.

VII. Parasites of Mosquito larvae recorded by various Authors.

Fungi: 1. W. G. Liston (1901) mentions an ectoparasitic fungus resembling *Trichophyton* (?) attacking *Anopheles* larvae in Ellichpur.

2. According to Vaney and Conte (quoted by Dyé, p. 15) *Culex pipiens* larvae are sometimes destroyed by a fungus *Botrytis bassiana* (which causes destruction of silkworms).

[1] Schröter, J. (1897). Chytridineae, Ancylistineae, Saprolegniineae in Engler and Prantl: *Die Natürlichen Pflanzenfamilien.*

3. Léger and Duboscq (1902) found in *Anopheles* larvae at Campo di Loro, Corsica, a filamentous fungus similar to that previously noticed by Perroncito.

4. Laveran (1902) describes a parasitic yeast discovered by him in the coelomic cavity of *Anopheles maculipennis* from Rio-Tinto (Spain). According to Dr Macdonald this yeast is probably transmitted from the larva to the adult.

5. A fungus *Coelomomyces stegomyiae* Keilin parasitic in the larva of *Stegomyia scutellaris* is described in the present paper; the parasitised host was found by Dr Lamborn at Kajang, Federated Malay States.

Bacteria: A bacterial parasite resembling *Leptotrix buccalis* was discovered by Perroncito (1899, quoted by Howard, Dyar and Knab) as pathogenic to *Anopheles maculipennis* in Turin, Italy. It infests the larva, passes into the pupa, and destroys the imago soon after it emerges.

Spirochaeta: *Spirochaeta culicis* Jaffé is described by Jaffé (1907) as infesting the alimentary canal of undetermined mosquito larvae collected near Berlin. A similar organism was found by Sergent and Sergent (1906) in *Anopheles maculipennis* larvae in Algeria.

Sporozoa: *Gregarina. Lankesteria culicis* (Ross 1898) Wenyon 1911 was found by R. Ross in mosquito larvae at Secunderabad and by Marchoux, Salimbeni and Simond (1903) in *Aëdes calopus* at Rio de Janeiro. The complete life history of this gregarine (from *St. fasciata*, Bagdad) we owe to Wenyon. Léger and Duboscq (1902) found a *Diplocystis* in mosquito larvae in Corsica.

Schizogregarina: A schizogregarine belonging to the genus *Caulleryella* Keilin (1914) has been recently described by Hesse (1918) under the name *Caulleryella anophelis* n.sp. parasitic in the alimentary canal of *Anopheles bifurcatus* larvae in Dauphiné, France.

Microsporidia: 1. *Nosema stegomyiae* was found by Marchoux, Salimbeni and Simond (1903) as pathogenic to *Stegomyia fasciata* larvae.

2. *Thelohania legeri* Hesse was described by Hesse (1904) as parasitic in *Anopheles maculipennis* larvae.

Thel. magna Kudo and *Thel. illinoisensis* Kudo were found by Kudo (1921) in the larvae of *Culex pipiens* and *Anopheles punctipennis* respectively.

Flagellata: Léger (1902) describes *Crithidia fasciculata* n.sp. parasitic in the gut of *Anopheles maculipennis*; the parasite is transmitted hereditarily.

Ciliata: A new ciliate, *Lambornella stegomyiae* Keilin was discovered by Lamborn in the body-cavity of *Stegomyia scutellaris* larvae, from Federated Malay States.

Nematoda: An *Agamomermis* was described by Stiles (1899, quoted by Howard, Dyar and Knab) from *Culex nemoralis* near Leipzig.

Dr Smith (quoted by Howard, Dyar and Knab, 1912, p. 163) records that in 1902 Dr H. P. Johns found the young of an intestinal worm in an *Anopheles* larva.

Gendre (1909) found larval nematodes at Labé, French Guinea, infesting *Stegomyia fasciata* larvae. It inhabits the body-cavity and leaves the host when the latter is ready to pupate; the larva then dies.

VIII. REFERENCES.

Dyé, L. (1905). Les parasites des Culicides. *Arch. de Parasitologie*, IX. 5–77.

Gendre, E. (1909). Sur les larves de *Mermis* parasites des larves du *Stegomyia fasciata*. *Bull. Soc. Path. Exot.* II. 106–108.

Hesse, E. (1904). *Thelohania legeri* n.sp. Microsporidie parasite des larves d'*Anopheles maculipennis* Meigen. *C. R. de la Soc. Biol.* LVII. 570–571.

—— (1904 *a*). Sur le développement de *Thelohania legeri* Hesse. *C. R. de la Soc. Biol.* LVII. 572–573.

—— (1918). *Caulleryella anophelis* n.sp. Schizogrégarine parasite des larves d'*Anopheles bifurcatus* L. *C. R. Ac. Sc.* CLXVI. 569–572.

Howard, L. O., Dyar, H. G. and Knab, F. (1912). The Mosquitoes of North and Central America and the West Indies, I. Carnegie Institution, Washington (see pp. 159–163).

Jaffé, J. (1907). *Spirochaeta culicis* nov. sp. *Arch. f. Protist.* IX. 100–107.

Keilin, D. (1914). Une nouvelle Schizogrégarine, *Caulleryella aphiochaeta*, n.g., n.sp., parasite intestinal d'une larve d'un Diptère cyclorhaphe (*Aphiochaeta rufipes* Meig.). *C. R. Soc. Biol.* LXXVI. 768–771.

—— (1921). On a new ciliate, *Lambornella stegomyiae* n.g., n.sp., parasitic in the body-cavity of the larvae of *Stegomyia scutellaris* Walker (Diptera, Nematocera, Culicidae). *Parasitology*, XIII. 216–224.

Kudo, R. (1921). Studies on *Microsporidia*, with special reference to those parasitic in Mosquitoes. *Journ. of Morphol.* XXXV. 153–193, Pls. I–V.

Lamborn, W. A. (1921). A Protozoon pathogenic to mosquito larvae. *Parasitology*, XIII. 213–215.

Laveran (1902). De quelques parasites des Culicides. *C. R. de la Soc. Biol.* LIV. 233–234.

Léger, L. (1902). Sur un flagellé parasite de l'*Anopheles maculipennis*. *C. R. de la Soc. Biol.* LIV. 354–356.

Léger, L. et Duboscq, O. (1902). Sur les larves d'*Anopheles* et leurs parasites en Corse. *C. R. de l'Assoc. fr. Av. Sc. Montauban*, pp. 703–704.

Liston, W. G. (1901). A year's experience of the habits of *Anopheles* in Ellichpur. *Ind. Med. Gazette*, XXXVI. 364.

Marchoux, E., Salimbeni, A. et Simond, P. L. (1903). La fièvre jaune. Rapport de la mission française. *Ann. Inst. Pasteur*, XVII. 665–731.

Perroncito, E. (1899). Sopra una speciale forma di micosi delle Zanzare. *Bolletino della R. Acad. di med. di Torino.*

Ross, R. (1906). Notes on the parasites of mosquitoes found in India between 1895 and 1899. *Journ. of Hygiene*, VI. 101–108.

Sergent, Edmont et Sergent, Etienne (1906). Sur un Flagellé nouveau de l'intestin des *Culex* et des *Stegomyia*, *Herpetomonas algeriense*. Sur un autre Flagellé et sur des *Spirochaete* de l'intestin des larves des Moustiques. *C. R. Soc. Biol.* LX. 291–293.

Stiles, Ch. W. (1903). A parasitic roundworm (*Agamomermis culicis* n.g., n.sp.) in American mosquitoes (*Culex sollicitans*). Hygienic Laboratory. *Public Health and Mar. Hosp. Service Bull.* XIII. 15–17.

Wenyon, C. M. (1911). Oriental Sore in Bagdad, together with observations on a gregarine in *Stegomyia fasciata*, the haemogregarine of dogs and the flagellates of house flies. *Parasitology*, IV. 273–344, Pls. XII–XVI.

STUDIES ON THE INTESTINAL PROTOZOA FOUND IN THE TERMITES OF JAPAN.

By MAKOTO KOIDZUMI,

Chief of the Laboratory of Medical Zoology, Government Institute of Science, Formosa, Japan.

(*From the Government Institute of Science, Formosa, and the National Institute for Medical Research, London.*)

(With Plates X to XV and 5 Text-figures.)

CONTENTS.

	PAGE
Introduction	235
Part I. Descriptive	236
1. Historical review	236
2. Material and Methods	240
3. Description of forms found in Japanese termites	243
Synopsis of genera and species	243
(1) Trichonympha Series	245
I. Genus *Trichonympha*	245
II. Genus *Pseudotrichonympha*	255
III. Genus *Teratonympha*	260
IV. Genus *Microspironympha*	267
V. Genus *Holomastigotoides*	270
VI. Genus *Spirotrichonympha*	275
VII. Genus *Holomastigotes*	277
(2) Pyrsonympha Series	278
I. Genus *Pyrsonympha*	281
II. Genus *Dinenympha*	289
Part II. General Discussion	295
1. Comparative Morphology	295
2. Classification	300
Bibliography	306
Description of Plates	308

INTRODUCTION.

The protozoa found in the intestines of termites constitute one of the most interesting but less illuminated fields in protozoology. The amount of work done by a number of authors in several different parts of the world is, however, already fairly large, especially valuable papers having appeared in recent years, so that some light has been thrown on the field. Nevertheless,

important questions regarding morphology, physiology, systematics, etc., still remain either insufficiently studied or almost untouched. Having myself been occupied for some years past with these interesting organisms, harboured by three species of termites in Japan proper and Formosa, and having made observations which seem to be of some interest, I propose in this paper to describe the chief results of my studies.

It was at the Government Institute for Infectious Diseases (Director: Professor S. Kitasato) in Tokyo that my studies were begun. After nearly a year, my post was changed to the Institute of Science, Government of Formosa, where my studies were continued for nearly two years. An account of my work, written in Japanese, has already been published in the Government Report of the Committee for the study of damage done by termites (1916 and 1917). I intended to publish a detailed report in English, but have been prevented from doing so until now. The publication of this paper has been rendered possible, owing to the unusual kindness of Mr Clifford Dobell, F.R.S., in improving my manuscript and preparing it for the press. He also made it possible for me to consult all the literature and gave me much valuable advice. This, together with my further studies made after the appearance of the first report, makes this paper differ in several respects from the original. Had it not been for Mr Dobell's hearty help, the present paper would have remained as an imperfect manuscript for some time longer. I therefore express my warmest thanks to Mr Dobell for his kindness. I have also to acknowledge my indebtedness to the staffs of the Institute for Infectious Diseases of Tokyo and the Institute of Science of Formosa, for their assistance during the course of my work, and to the Medical Research Council for affording me laboratory accommodation and aid which enabled me to complete my studies in London.

PART I. DESCRIPTIVE.

1. HISTORICAL REVIEW.

The first author who mentioned the peculiar intestinal protozoa of termites was apparently Lespès (1856), who found "Infusoria" in *Leucotermes lucifugus*. In two papers, which appeared in 1877 and 1881, Leidy gave the first detailed descriptions of three forms which he had discovered in *Termes flavipes* of North America, referring them to three genera, viz. *Trichonympha*, *Pyrsonympha*, and *Dinenympha*. A very short note by Seip (1881), recording that he had found organisms like those described in Leidy's paper (1877) in termites of New Jersey, appeared shortly afterwards. In 1885 appeared Saville Kent's short paper dealing with species found in a Tasmanian termite, and also with the forms studied by Leidy. Subsequently Grassi and his coworkers made valuable contributions to the advancement of our knowledge of the group. After publishing several short notes in 1885, 1888, and 1892, in a famous work on the termites published in 1893 conjointly with Sandias,

Grassi gave revised descriptions of the organisms studied by him up to that date. The host insects which he had examined were *Calotermes flavicollis* and *Termes lucifugus*, and the organisms were referred to seven genera, viz. *Joenia*, *Trichonympha*, *Microjoenia*, *Monocercomonas*, *Dinenympha*, *Pyrsonympha*, and *Holomastigotes*, three of these being marked as new[1].

Three years prior to the appearance of the above work of Grassi and Sandias, Simmons (1890) described the organisms harboured by termites in Calcutta (genera and species not mentioned). He did not give names to the intestinal protozoa, and his descriptions are not sufficiently detailed for it to be possible now to determine their systematic position. His paper is of interest, however, as it constitutes the first record of the occurrence of protozoa in Asiatic termites. In 1891, Frenzel described a form referred to a new genus, *Leidyonella*, found in *Eutermes inquilinus* of the Argentine. A work by Porter, dealing with the forms previously studied by Leidy, appeared in 1897, and largely enriched our knowledge of their structure.

All the above-mentioned works were concerned with morphology alone, and no account of the mode of multiplication and development, based upon accurate observations, was given until 1904, when there appeared a description of the multiplication of *Joenia* by Grassi and Foà, and of *Trichonympha* by Foà. In the following year, Foà (1905) described two forms belonging to new genera, *Calonympha* and *Devescovina*. In 1910 a form wrongly referred to a new genus, *Lophophora*, was reported from Italy by Comes (*vide infra*), and Dobell also described a form from Ceylon, establishing a new genus *Gymnonympha* for it. In the same year appeared a work by Hartmann (1910), embodying the results of his studies on the organisms harboured by *Coptotermes hartmanni* of Brazil, and some theoretical considerations based upon his studies. He distinguished three forms and interpreted them as the young, the male, and the female of a species called *Trichonympha hertwigi*. With regard to the position of these peculiar organisms in the system of the protozoa, two different views prevailed at that time, some authors classifying them under the Mastigophora, while others placed them among the Ciliata. In the above-mentioned work, Hartmann attempted to establish a new division, Trichonymphida, independent of both the Mastigophora and the Ciliata. This work, however, was soon followed by a paper by Grassi and Foà (1911), pointing out that Hartmann's memoir contains many careless judgments and erroneous conceptions. They disapproved of Hartmann's view of the relationship of the three types which he described, and Grassi established two new genera, *Pseudotrichonympha* and *Holomastigotoides*, for the forms taken by Hartmann for "males" and "females" respectively ("male" and "female" misprinted in reverse in the original, and corrected afterwards). They did not, moreover, accept the view of Hartmann as regards the systematic position, and insisted upon their old classification of the organisms among the flagellates. In this

[1] Namely, *Joenia*, *Microjoenia*, and *Holomastigotes*. But it should be noted that the first of these was really named by Grassi in 1885, and the other two in 1892.

paper, also, they made revisions of the hitherto described genera and species, and established four new genera, besides the two above-mentioned, viz. *Eulophomonas, Mesojoenia, Spirotrichonympha, Microrhopalodina.*

Janicki published a beautiful work on *Lophomonas* in 1910. *Lophomonas* is the only known genus closely allied to the organisms under consideration which occurs in insects other than termites—it being found in the intestine of the cockroach. In two works which appeared in the following years (1911 and 1912), he dealt with the structure and multiplication of flagellates in the intestines of cockroaches and termites. The termites studied by him were two species of *Calotermes* (*C. castaneus* from Hawaii and *C. grassii* from Chile), and he established two new genera for their protozoa, viz., *Parajoenia* and *Stephanonympha.* In 1915 Janicki published a larger work, embodying the results of his further studies on the organisms harboured by the above two species of termites. In this paper he described seven forms, referred to the five genera *Devescovina, Parajoenia, Stephanonympha, Oxymonas, Foaina* (the last two being new), and several flagellates of uncertain genera, together with his studies on division, his views on the systematics of the group, and some other items.

During the years 1910–1913, Bugnion (partly with his co-workers, Popoff and Ferrière) published several papers dealing with the termites of Ceylon and the casts of termites generally. In these works, descriptions and figures of several intestinal protozoa were given, which are, however, not calculated to enrich our knowledge of these organisms. In 1912 appeared two works by Comes, dealing with the development of *Pyrsonympha* (a preliminary note for one of these (1910 *a*) had appeared two years before), and in 1914 another paper describing a species of "*Monocercomonas*" found in *Termes lucifugus* and *Calotermes flavicollis.* In all of the papers by Comes there are many mistakes in identification of genera and wrong understandings of several forms.

In 1915 Zulueta gave a description of nuclear division in a species of "*Dinenympha*" studied in Spain. Dogiel (1916) soon afterwards described four species of protozoa harboured by a *Rhinotermes* (?) from Uganda, referring them to three genera, viz. *Trichomonas* (*Tetratrichomonas*), *Gigantomonas*, and *Myxomonas*, the latter two being newly established. In 1916 also appeared a work by França, dealing with organisms referred to two genera, *Trichonympha* and *Leidya* (gen. nov.), in the *Leucotermes* of Portugal; and three more papers (França, 1918, 1918 *a*, 1918 *b*) were published later, which embodied the results of his further studies and his opinions on the classification of these forms. The two genera (*Leidya* and *Caduceia*) established by him, however, are probably synonyms of *Spirotrichonympha* and *Devescovina* respectively.

In 1917 appeared a large work by Grassi, which contains the descriptions of many forms found in *Porotermes adamsoni* from Australia, *Epicalotermes aethiopicus* from Eritrea, *Coptotermes sjöstedti* from French Guinea, *Copto-*

termes lacteus from Australia, *Schedorhinotermes putorius* from Australia, and *Schedorhinotermes intermedius* from French Guinea. He referred the protozoa to 13 genera, 18 species, and 5 varieties—5 of the 13 genera, viz. *Joenina*, *Staurojoenia*, *Spirotrichonymphella*, *Pseudotrypanosoma*, and *Diplonympha*, being newly established ones.

In a work on the structure and biology of *Archotermopsis* of India, published in 1919, Imms gave descriptions of the intestinal protozoa harboured by that termite. Further studies of these organisms are being carried on by Cutler, who obtained a stock of the living termites from Imms, and two papers (1919 and 1920) have already been published, embodying his studies on species of *Trichomonas* (*Ditrichomonas*) and *Joenopsis* (gen. nov.). Kofoid and Swezy published in 1919 a series of papers dealing with the structure and mode of multiplication of four species of intestinal protozoa harboured by *Termopsis angusticollis* of California. Each species was referred to a different genus, viz. *Streblomastix*, *Trichomitus*, *Trichonympha* and *Leidyopsis*, the first and the last being new.

The physiological relationship between the termites and their intestinal protozoa is a highly important and deeply interesting subject. Do the organisms live in the intestine parasitically, commensally, or symbiotically, as different authors have supposed? A study of this question has been made by Buscalioni and Comes (1910). They examined the reactions to chemical reagents of each region of the body of the protozoa, to determine the fate of the ingested wood fibres, and reported their results in detail. Imms (1919) has also devoted one chapter of his work to the consideration of some physiological questions.

A note by Buttel-Reepen (1914) recording the results of his examination of many genera of termites in Java, Sumatra, Malacca, and Ceylon, to determine the presence or absence of intestinal protozoa, will be suggestive for further studies; but his work, though adding to our knowledge of the distribution of these protozoa, contains nothing else of interest for the present paper.

A list of genera hitherto described will be given here. The genera established for the organisms harboured exclusively by termites, the type species of these genera, and their synonyms, are as follows:

GENUS.	TYPE SPECIES.
Trichonympha Leidy, 1877.	*T. agilis* Leidy.
Pyrsonympha Leidy, 1877.	*P. vertens* Leidy.
Dinenympha Leidy, 1877.	*D. gracilis* Leidy.
Joenia Grassi, 1885.	*J. annectens* Grassi.
Leidyonella Frenzel, 1891.	*L. cordubensis* Frenzel.
Microjoenia Grassi, 1892.	*M. hexamitoides* Grassi.
Holomastigotes Grassi, 1892.	*H. elongatum* Grassi.
Devescovina Foà, 1905.	*D. striata* Foà.
Calonympha Foà, 1905.	*C. grassii* Foà.
Gymnonympha Dobell, 1910.	*G. zeylanica* Dobell.

Genus.	Type Species.
Lophophora Comes, 1910.	*L. vacuolata* Comes.
= *Pyrsonympha* Leidy.	
Microrhopalodina Grassi, 1911.	*M. enflata* Grassi.
Eulophomonas Grassi, 1911.	*E. calotermitis* Grassi.
Mesojoenia Grassi, 1911.	*M. decipiens* Grassi.
Pseudotrichonympha Grassi, 1911.	*P. hertwigi* (Hartmann) Grassi.
Spirotrichonympha Grassi, 1911.	*S. flagellata* Grassi, 1893.
Holomastigotoides Grassi, 1911.	*H. hertwigi* (Hartmann) Grassi.
Parajoenia Janicki, 1911.	*P. grassii* Janicki.
Stephanonympha Janicki, 1911.	*S. silvestrii* Janicki.
Oxymonas Janicki, 1911.	*O. granulosa* Janicki.
Foaina Janicki, 1911.	*F. gracilis* Janicki.
Leidya França, 1916.	*L. metchnikovi* França.
= *Spirotrichonympha* Grassi.	
Gigantomonas Dogiel, 1916.	*G. herculea* Dogiel.
Myxomonas Dogiel, 1916.	*M. polymorpha* Dogiel.
Joenina Grassi, 1917.	*J. pulchella* Grassi.
Staurojoenia Grassi, 1917.	*S. mirabilis* Grassi.
Spirotrichonymphella Grassi, 1917.	*S. pudibunda* Grassi.
Macrotrichomonas Grassi, 1917.	*M. pulchra* Grassi.
Pseudotrypanosoma Grassi, 1917.	*Ps. giganteum* Grassi.
Diplonympha Grassi, 1917.	*D. foae* Grassi.
Caduceia França, 1918.	*C. theobromae* França.
= *Devescovina* Foà.	
Streblomastix Kofoid et Swezy, 1919.	*S. strix* Kofoid et Swezy.
Leidyopsis Kofoid et Swezy, 1919.	*L. sphærica* Kofoid et Swezy.
Joenopsis Cutler, 1920.	*J. polytricha* Cutler.

Other genera of protozoa of which species have been reported in the intestines of termites are the following:

Flagellata { *Monocercomonas* [*M. termitis* Grassi].
Trichomonas (*Ditrichomonas*) [*T.* (*D.*) *termitis* (Imms) Cutler].
„ (*Tetratrichomonas*) [*T.* (*D.*) *termitidis* (*sic*) and *macrostoma* Dogiel].
Trichomitus [*T. termitidis* (*sic*) Kofoid et Swezy].

Ciliata .....*Nyctotherus* [*N. termitis* Dobell].

Sporozoa ..."*Gregarina*" ["*G.*" *termitis* Leidy].

2. MATERIAL AND METHODS.

The species of termites recorded as occurring in Japan and its territories are rather large in number (16 species according to M. Oshima, and 13 or 15 species according to Holmgren and Hozawa). As a matter of fact, the termite fauna is remarkably rich in the subtropical and tropical regions, so that all of the reported species are found in Formosa[1], while in Japan proper[2]

[1] The island of Formosa lies at the southern extremity of the Empire of Japan, extending from 120° 2′ to 122° 6′ E. of Greenwich, and in latitude from 21° 45′ to 25° 37′ N.; the area covering 13,908 square miles.

[2] Conventionally we divide the Empire into Japan proper, Formosa, Saghalien, and Chosen (Korea)—Chosen being a peninsula of the continent, Saghalien an island at the northern extremity, and Japan proper the other islands forming the main portion of the Empire.

there occur only two species, viz. *Leucotermes* (*Reticulitermes*) *speratus* and *Coptotermes formosanus*. Though the termites of Formosa are so rich in species, the great majority of these species are not of common occurrence, but are rare or locally distributed, and only a few species are found commonly throughout the island. The species most commonly found in Formosa are *Coptotermes formosanus* and *Odontotermes formosanus*. A species of *Leucotermes* is also more or less frequently found in Formosa, and referred to different species by different authors. M. Oshima distinguished it from *Leucotermes speratus*, saying that although it is scarcely possible to draw a sharp line of distinction between the imagines, yet the soldiers display some distinct differences which justify their separation. S. Yano, S. Hozawa, and Holmgren, on the other hand, persist in the belief that they should be united into one. Not being an entomologist, I myself will not express an opinion on this species question. As regards their protozoa, however, I find that distinct differences are recognizable between those of *Leucotermes speratus* of Japan proper and those of the disputed species of Formosa. Thus I feel it convenient for my description to let the Formosan species have its own name, and I prefer to call it by the name given by M. Oshima, *Leucotermes* (*Reticulitermes*) *flaviceps*.

In carrying out my studies both in Tokyo and Formosa, I was fortunate in obtaining an ample supply of living specimens of four species of the host insects. While at Tokyo, my studies were done on the forms harboured by *Leucotermes speratus*, collected at several localities in Japan proper. On going afterwards to Formosa, three different species, *Coptotermes formosanus*, *Leucotermes flaviceps*, and *Odontotermes formosanus*, came into my hands. Moreover, I have had an opportunity of examining another species of rather rare occurrence, *Capritermes nitobei*. In *Odontotermes formosanus* and *Capritermes nitobei*, however, none of the protozoa under discussion were found. While working at Tokyo, I was unable to obtain any specimens of *Coptotermes formosanus*; but shortly before finishing this study I made a trip to Kyushu, for the purpose of studying *Coptotermes formosanus* and *Leucotermes speratus* in that southern district of Japan proper.

In all the three species of termites studied by me (*Leucotermes speratus*, *L. flaviceps*, and *Coptotermes formosanus*), the protozoa are found in equally large numbers, and almost the entire cavity of the intestine of every individual is found filled with them, excepting the oesophagus and the proventriculus. The Malpighian tubules also were found frequently invaded by some of the forms with slender bodies. Young termites, lately hatched, have their intestines free from protozoa; but as they begin to take food, the protozoa seem quickly to invade them, for individuals measuring only three millimeters in length are usually found rather heavily infected.

In the workers the intestine is more developed than in the other casts, and is found almost filled with protozoa, food substances being rather small in volume as compared with the contained organisms. In the soldier and

the nymph the intestine is rather slender, but is also almost filled with protozoa. In the imago the condition is variable; in some individuals the intestine is quite free from protozoa while in others they are present in rather large numbers. Regarding the infections of imagines, my experience is not sufficiently extensive, and further investigations are needed.

The protozoa found in every individual termite are abundant, not only in number, but also in species. Almost all the kinds proper to a particular species of host are commonly found in every individual of that species. No differences are recognizable among the protozoa found in workers, soldiers, and nymphs, and all kinds usually occur with equal frequency in the above casts. In imagines which contain protozoa, the conditions seem also identical.

The fact that the protozoa are present with such frequency is probably to be explained by the habits of the termites themselves. They live in communities, and, as is well known, eat one another's faeces. The space in which they live is usually dark and moist, so that the faeces must usually remain damp, and the protozoa passed out with them, even though not encysted, may thus remain alive for some time. It seems probable also that the same habits explain the fact that the young termites become heavily infested in very early stages of their development.

As mentioned in the foregoing historical review, the physiological relationship between these protozoa and their insect hosts is a subject of profound interest and great importance. For the consideration of the rôle played by the protozoa in the digestion of wood fibres in the intestine, the habits of all termites, both those harbouring protozoa and those free from them, must be carefully investigated.

I may add here a brief note on the habits of the species studied by myself. *Leucotermes speratus* and *L. flaviceps*, harbouring *Trichonympha*, *Teratonympha*, *Holomastigotes*, *Microspironympha*, and *Pyrsonympha*, are species doing some damage to wooden structures. These species form irregular cavities and burrows under or in rotten boards, timbers, decayed logs, fallen trees, and similar material. *Coptotermes formosanus*, harbouring *Pseudotrichonympha*, *Holomastigotoides*, and *Spirotrichonympha*, is the most destructive species in Japan, especially in Formosa. Wooden buildings, furniture, logs, and decayed portions of living trees are severely attacked by this species. *Odontotermes formosanus*, harbouring none of these protozoa, sometimes attacks also timber and trees, though not causing such extensive damage. Soft materials, however, such as the stems and roots of sugar cane are often destroyed by this species. *Capritermes nitobei* in which I did not find any of the protozoa in question—though my material was not abundant—has similar habits to those of *Odontotermes*.

The fluid medium chiefly used for the observation of living organisms was a 0·3–0·4 per cent. solution of sodium chloride. The same fluid was also used for diluting the gut contents in the preparation of fixed smears. Observations of the living organisms were found most important in studying these forms;

and several minute points of structure, which can hardly be made out in fixed and stained preparations, are clearly observable in fresh specimens. Dark-ground illumination and vital staining methods were tried and proved very fruitful in many cases. For the fixative, Schaudinn's solution was almost exclusively used, and the smears, prepared on cover-glasses, were fixed by dropping them face downwards on the fixative. The most excellent and frequently used stains were iron-haematoxylin methods. I used in most cases 2 per cent. solution of iron-alum and ripe alcoholic solution of haematoxylin. Giemsa's fluid, thionin, fuchsin, and other aniline dyes were also used and found useful in several cases. Some bacteriological stains were also used for the study of special structures. Preparations fixed and cut into sections were also made, but were found unsuitable for morphological study; and my experience taught me that this method is apt to lead to erroneous judgments. Thus total preparations were chiefly relied upon, except for the purpose of studying the position of the protozoa in the intestine and their relation to its wall.

3. DESCRIPTION OF FORMS FOUND IN JAPANESE TERMITES.

The present paper embodies the results of my studies of forms referred to eight genera. Six of these eight are genera known already to occur exclusively in the intestines of termites; the other two are new, and belong to the same group. Other protozoa were also found in the termites studied. These forms are of smaller size, and while some are trichomonad types, others are of peculiar organization. My studies of these forms are, however, not complete, so that I shall refrain in this paper from making any further mention of them.

Before entering into a description of each form, a brief synopsis of the genera and species will be given here, as I think it will be helpful to the reader.

SYNOPSIS OF GENERA AND SPECIES.

Our forms are conveniently divided into two main groups, which may be called (1) the *Trichonympha* series and (2) the *Pyrsonympha* series. These groups are not to be taken as constituting families or higher orders: they are used here merely for convenience in description. The classification of the organisms contained in them will be discussed later (p. 300). The first series contains forms provided with a very large number of flagella, and the second those with only four or eight flagellar cords. The species belonging to the former series are found in both *Coptotermes* and *Leucotermes*, while those of the latter are limited to *Leucotermes*.

(1) *Trichonympha* series.

Six genera are placed in this series. It may be subdivided into two groups, of which *Trichonympha* and *Holomastigotoides* may be regarded as the representative types.

A. *Trichonympha* group.

Large forms of complicated organization, provided with numerous flagella. Three genera are distinguished:

I. *Trichonympha* Leidy.

Body oval or lanceolate, its posterior region destitute of flagella. I distinguish two varieties of a single species:

(*a*) *T. agilis* var. *japonica* var. nov.[1] (in *Leucotermes speratus*);
(*b*) *T. agilis* var. *formosana* var. nov. (in *Leucotermes flaviceps*).

II. *Pseudotrichonympha* Grassi.

Very large, and spindle-shaped; the posterior region of the body provided with flagella arranged in spiral rows. One species:

P. grassii sp. nov. (in *Coptotermes formosanus*).

III. *Teratonympha* gen. nov.

Very large, long and club-shaped. Numerous transverse ridges on the surface give the organism a segmented appearance, so that it resembles a cestode. Flagella arranged in transverse rows. One species and one variety:

(*a*) *T. mirabilis* gen. nov. et sp. nov. (in *Leucotermes speratus*);
(*b*) *T. mirabilis* var. *formosana* var. nov. (in *Leucotermes flaviceps*).

B. *Holomastigotoides* group.

Large or small forms, less complicated than the preceding. Flagella arising in rows, which have a common point of origin at the anterior extremity and wind spirally backwards. Four genera are distinguished:

IV. *Microspironympha* gen. nov.

Body small, and spindle-shaped or piriform. A tubular structure connects the anterior tip of the body and the nucleus. Flagellar bands few, a rather large portion of the posterior region being free from them. One species:

M. porteri gen. nov. et sp. nov. (in *Leucotermes flaviceps*).

V. *Holomastigotoides* Grassi.

Very large, piriform or ovoid, with flagellar bands distributed densely over almost the entire surface of the body. One species:

H. hartmanni sp. nov. (in *Coptotermes formosanus*).

[1] Throughout this paper I have designated the new forms which I have found in Japanese termites as "var. nov.," "sp. nov.," etc. The majority of these have, however, already been described and named in my earlier papers (1916, 1917). But these were written in Japanese, and were therefore inaccessible to most zoologists; and I am, moreover, uncertain whether the names can be regarded as having any status in international nomenclature. I have therefore marked all such names as technically "new" in the present memoir.

VI. *Spirotrichonympha* Grassi.

Body conical and rather small. Flagellar bands fewer, distributed over almost the whole of the body. The bands are peculiar in their disposition, being situated somewhat deeply in the protoplasm. One species:

S. leidyi sp. nov. (in *Coptotermes formosanus*).

VII. *Holomastigotes* Grassi.

Body small and spindle-shaped, its surface spirally ridged; flagella arising in the grooves behind the ridges. One species:

H. elongatum Grassi (?) (in *Leucotermes speratus* and *L. flaviceps*).

(2) *Pyrsonympha* series.

The body large or small, and club-shaped, fusiform, or piriform. An axial filament, fixed anteriorly, runs down the body. Four or eight slender cords start at the tip of the filament and run spirally backwards, fixed on the body wall, to the posterior extremity, where they emerge as free flagella.

A single genus, *Pyrsonympha*, divided into two subgenera, *Pyrsonympha* and *Dinenympha*. A synopsis of the subgenera and the species will be given at the beginning of the description of these forms (p. 281).

(1) Trichonympha Series.

I. *Trichonympha.*

This is one of the three oldest genera established in 1877 by Leidy for the American species. Under this genus six species have hitherto been described, viz. *T. agilis*, *T. leidyi*, *T. minor*, *T. magna*, *T. hertwigi*, and *T. campanula*. *T. agilis* is the species described by Leidy (1877 and 1881) in America, and its behaviour and structure, as far as they could be studied in the living condition, were carefully observed and accurately described and figured by him. Some 20 years later, Porter's work on the same species appeared (1897), and threw much new light upon the finer structures. This species was also found in Italy, and was described by Grassi[1] in his famous work with Sandias (1893) on the termites. At first the Italian forms were considered to be identical with the American, but afterwards two types, *major* and *minor*, were distinguished by Foà (1904). The latter type was subsequently made by Grassi (1911) into a species, *T. minor*. In recent years several reports dealing with the forms belonging to this genus have been published. França (1916 and 1918) described the structure of an organism, identified as *T. agilis* by him, harboured by Portuguese termites. In 1917 a voluminous paper by Grassi appeared, in which large numbers of species of many genera, harboured by termites from various localities, were dealt with; and one new species of this genus—*T. magna*—was described from an Australian termite. One more report was published by Kofoid and Swezy in 1919. They described a new

[1] According to Grassi (1885) the organism was discovered in Italy by Condorelli.

species, *T. campanula*, harboured by *Termopsis* in North America, and its structure and mode of multiplication were discoursed in detail.

In papers which appeared in earlier years, the generic name *Trichonympha* was erroneously applied to various forms with quite different characteristics. For instance, a form briefly described by Kent (1885), shortly after the appearance of Leidy's work, and named *T. leidyi*, is doubtless a member of another genus. More recently, this generic name was applied by Hartmann (1910) to the organisms—"*T.*" *hertwigi*—harboured by Brazilian termites. But these present peculiarities proper to more than two other genera, and Grassi soon established two new genera for them.

Forms belonging to this genus are found in *Leucotermes* of both Japan proper and Formosa. Our forms show a rather close resemblance to *T. agilis*, described by the American authors, but some points of difference are definitely recognizable among them. There are, moreover, some distinct differences between those of *Leucotermes speratus* of Japan proper and those of *Leucotermes flaviceps* of Formosa. The differences found among the three forms, however, are not judged to be sufficiently great to make them separate species, and I propose to treat our forms as varieties of the American species, calling them *T. agilis* var. *japonica* and var. *formosana*.

(1) *Trichonympha agilis* var. *japonica* var. nov. (Plate X, figs. 1–4).

The body assumes an oval shape, slightly narrowed at the anterior end and evenly rounded at the posterior. The dimensions are usually 70–90 μ in length, and 40–70 μ in breadth. The body is clearly differentiated into anterior and posterior regions ("head" and "body" according to Leidy's terminology). The front end of the former is drawn out into a nipple-like prominence. The anterior region, occupying some one-fourth of the whole length, is quite different in structure from the rest of the body; its wall being remarkably thick, and its endoplasm seeming denser, so that no marked changes in shape are observed in this region. In the posterior region, on the other hand, the body wall is not so thick and the endoplasm appears less dense, so that it is rather metabolic. Porter called the anterior eminence the "nipple" and the rest of the "head" the "bell-shaped region," or simply the "bell." The terms "nipple" and "bell" will be used in this paper. In *Trichonympha agilis*, the anterior region is of about the same length as the posterior, or a little shorter; that is to say the bell is markedly larger than that of our variety. In *T. campanula* the bell is still larger.

One of the conspicuous features of the forms of this genus is the coat of flagella, thickly distributed on the anterior region. They are very closely set on both the nipple and the bell, excepting only the specially differentiated anterior tip. These flagella are directed backwards and increase in length towards the boundary of the anterior and the posterior regions. The longest ones attain so remarkable a length as to extend beyond the posterior extremity

of the body. The flagella are directed backwards, applied more or less closely on the body wall, and are regularly arranged, so that they give the surface of the body a very beautiful appearance of parallel stripes.

The nipple is a very complicated structure (Pl. X, fig. 13). It has the shape of a cone with an evenly rounded top, and rests on the anterior border of the bell. Its height is about 10 μ and its width is about two-thirds of the height. It is remarkably rigid in consistency, so that it hardly shows any change of shape, even when the anterior portion of the body is swung actively in all directions. Four parts can be distinguished in the nipple, which were named by Porter as follows: (1) the axial rod: (2) the inner layer; and (3) the outer layer, surrounding the axial rod; and (4) the "cap" at the top of the nipple. I propose to follow the above terminology, altering only "axial rod" into "axial core." Of the above four parts, the axial core and the inner layer come together so as to form the axis of the nipple. The inner layer encircles the greater part of the axial core, leaving uncovered only a small portion of the apical part, which expands into a knob-like enlargement. The columnar part of the axial core is of almost uniform width, but slightly narrowed towards the anterior end, and having a constriction behind the knob. The knob and the constricted portion are quite structureless and refractive; while the columnar part is not homogeneous, but consists of two parts, namely, an outer wall of almost uniform thickness, and its contents—the latter appearing slightly granular and being continuous behind with the endoplasm of the bell. The inner layer is homogeneous in structure and refringent. The outer layer covers the inner layer throughout its whole extent. It is widest at the base, and is there about twice as thick as the inner layer. It gradually decreases in thickness towards the front end, where it becomes very much tapered and terminates under the knob of the axial core, together with the inner layer. Thus the above three parts assume anteriorly, as a whole, the shape of a dome with a knob resting on its top.

The "cap" is a structure with the shape of a hemispherical bowl: it occupies the top of the nipple, with its margin resting on the sloping surface of the outer layer. The space confined by the cap in front of the above described dome-shaped part is filled with a homogeneous substance. This part of the nipple is very rigid in this variety, and displays no recognizable alterations of shape. The outer layer is traversed by the fine flagella, which reach as far as the outer surface of the inner layer. The flagella are so numerous and densely set, that, both in top view as well as in side view, they appear to occupy the greater part of the outer layer. The most anterior flagella pass downwards along the sloped surface of the frontal part of this layer, and become free at the place where the margin of the cap rests upon it. The flagella are not of uniform length. Those situated at the tip are the shortest, and are nearly as long as the nipple. They gradually increase in length towards the base of the nipple, where they become twice as long as the height of the latter.

The knob and the narrow portion of the axial core stain intensely with iron-haematoxylin, as well as with eosin. The wall of the columnar part also stains deeply, while its contents do not take any dyes at all, but contain deeply stained granules. The inner layer stains only slightly or not at all, while the outer layer appears rather deeply stained. In well-stained preparations further differentiation, which has been overlooked by previous authors, is discernible in the inner layer. This consists of a zone, somewhat darkly stained, close to the axial core, and measuring some one-fourth of the layer in its thickness. The boundary between the inner and the outer layers stains intensely with haematoxylin, thionin, fuchsin, and other dyes. The existence of such a zone, and the structure of the axial core and the inner layer, afford us, together with the result of studies of living specimens, strong evidence for believing that the flagella arise from this zone.

It has already been remarked that the bell is distinctly differentiated from the rest of the body, especially as regards its wall. The wall is very thick and one can easily detect the outer and the inner layers both in the living animal and in stained preparations. These layers are of the same structure as the corresponding layers enclosing the axial core, and are apparently continuous with them. There is, however, as pointed out by previous authors, a circular fissure ("citartrosi" of Grassi) encircling the base of the nipple and reaching the base of the axial core. It is, therefore, by means of the axial core only that the nipple is connected with the body. As in the nipple, the outer layer of the bell is much thicker and stains more deeply than the inner layer. The latter stains feebly or not at all, excepting a zone at the base, which stains more deeply like the corresponding zone in the nipple. In the living animal, the inner layer at once attracts the observer's attention as a transparent zone. In stained preparations the deeply stained zone at the boundary between the two layers is much more conspicuous than in the nipple. The numerous long flagella undoubtedly arise from this zone and traverse the outer layer.

The descriptions of the structure of the nipple and the wall of the bell in *Trichonympha agilis*, given by previous authors, do not agree with the results of my observations in several points. As for the axial core, to begin with, the descriptions of Porter (1897) almost agree with mine, except that he describes a slight difference in its shape. Grassi's descriptions seem mostly to agree with mine, but differ in the following point. According to him, the anterior knob is not solid, but consists of two parts, namely, wall and contents, just as in the columnar part; the contents of both parts being the same, and continuous with each other. The axial core of the Portuguese species, described by França, is of a quite peculiar structure. He described (1916) and illustrated (1918) this structure as consisting of a mushroom-shaped body and a projecting transverse disc, connected with each other by a commissure. He did not mention any differentiation of its wall and contents. According to Kofoid and Swezy (1919), the structure of the nipple is very peculiar in *T. campanula*. There is no deeply stainable tubular wall, but several longi-

tudinal strands of deeply stainable substance are observed surrounding the columnar core, corresponding to the contents of the axial core in *T. agilis*. At the anterior end, there is no structure corresponding to the knob in *T. agilis*; and there is either only a ring of deeply stainable substance at the tip, or else the strands are simply united together at the anterior end. The descriptions of the layers surrounding the axial core, of the wall of the bell, and of the flagella, given by previous authors, are also diversified. According to Porter, the inner layer of the bell is marked with fine lines perpendicular to the surface of the body wall, and he took these lines to be traces of traversing flagella.

Grassi's statements are, to me, very confusing. He describes several different appearances seen by him, and does not give any interpretations to reconcile his diverse observations. His opinion seems, however, to be as follows. The inner layer is liquid, and the basal granules of the flagella are found at the base of this layer, situated on longitudinal ridges lying directly under it. In some of his figures, the ridges are shown as forming a distinct layer. According to him, the flagella are sometimes provided with a node or granule at the outer boundary of the layer. França described the flagella as arising from the surface of the axial core; those of the nipple from the mushroom-shaped body and those of the bell from the disk-like part. A differentiation into layers, both in the nipple and in the bell, seems not to be recognized by him; but the existence of such a differentiation cannot be doubted. The body wall of *T. campanula* is figured by Kofoid and Swezy (1919) in various ways and their descriptions are not easy to understand; but, according to Professor Kofoid's personal explanation—which he kindly gave me in London—the wall of the bell consists of four layers. The flagella arise at the outer boundary of the innermost layer (drawn homogeneous in some figures and granular in others), and each flagellum has a node or granule at the middle of the portion embedded in the body wall. The outer part of the body wall, traversed by the flagella, is divided by an alveolar layer into outer and middle layers.

There are thus many points of disagreement among the statements of previous authors, and between theirs and mine; and I cannot but doubt the accuracy of their observations. It will be worthy of mention, here, that some of the above views were founded upon observations made on material fixed and cut into sections, which I believe, from my own experience, are apt to lead us to erroneous results. The space encircling the base of the nipple is described by Grassi, and Kofoid and Swezy, as rather spacious. But it is usually a narrow slit in my organisms, and it was only in specimens badly fixed or cut into sections that I found it wide and distinct.

In the posterior region, the body wall is not thick, though it is fairly rigid and stains rather deeply. No clear layer corresponding to the inner layer of the anterior region is here recognizable.

The endoplasm is finely granular, homogeneous, and appears slightly grey

in colour during life. Beneath the body wall is seen a zone of particularly coarse and deeply stainable granules. It is specially dense under the thick wall of the bell. This layer of granules lies beneath the body wall, and it is found in good preparations that they are embedded in a very thin layer of transparent and structureless substance lying immediately under the body wall.

The nucleus is invariably situated in the median line and a little anterior to the middle point. It does not lie freely in the endoplasm, but has a special structure keeping it in connexion with the body wall. This interesting structure was called by Grassi the "cestello" or "little basket," and I propose to use the term "corbule" for it in this paper. The corbule has the following structure. In living as well as in stained preparations, a distinct zone of granules, identical with those distributed beneath the body wall, is seen on both sides of the nucleus, connecting its posterior surface with the wall of the bell. Careful study reveals the presence of a thin membranous structure resembling an inverted cone or a hemispherical bowl, with the granules on its internal surface; and it can be seen that the nucleus is situated at its bottom. At the edge of the bell the granules lining the membrane pass gradually over to the well-developed layer of similar granules under the body wall. In the peripheral part of this structure, the granules are distributed as deeply as under the wall of the bell; and the outline of the structure itself is so sharp on its external surface that the existence of a distinct membrane seems quite certain. Towards the nucleus, however, the membrane becomes gradually indistinct, and it is often quite obscure in the immediate vicinity of the nucleus. The granules are commonly distributed uniformly, both under the wall of the bell and on the membrane, but in some individuals a regular alternate arrangement of dense and thin bands of granules is noticeable (Pl. X, fig. 4). The bands are usually of almost uniform width and are found, in well-developed cases, extending from the middle part of the bell to the vicinity of the nucleus.

The shape of the corbule is more or less variable. Usually it assumes, in my organisms, the shape of a hemispherical bowl, with the nucleus situated just at its bottom; neither the membrane nor any trace of the granules being discernible behind the nucleus (Pl. X, fig. 1). In some individuals, however, it assumes the shape of an inverted cone, touching the nucleus tangentially; and the membrane is then visible behind the nucleus, with granules attached to it, and sometimes appears fibrous (Pl. X, figs. 2 and 3). In some other individuals the structure is indistinct in the vicinity of the nucleus, and an irregular mass of granules is seen around the latter. In my organisms still another structure, keeping the nucleus in connexion with another part of the body, is recognizable. This is a columnar mass of somewhat indistinct contour, extending perpendicularly from the apex of the bell to the nucleus (Pl. X, fig. 3). It apparently consists of the same kind of granules as those under the wall of the bell and on the corbule, but I am of the opinion that there is really

a matrix of special protoplasm—which I think to be the same as that of the corbule—in which the granules are embedded. The columnar structure is commonly thick and distinct at the anterior part and becomes gradually indistinct towards the nucleus. In some individuals the columnar structure can be traced clearly to the anterior surface of the nucleus, but appears in some others to end before reaching it. The endoplasm in front of and behind the corbule is not continuous. In the posterior part it is less dense and contains various kinds of food débris.

In the American species Leidy distinguished the "head endosarc" from the "body endosarc," but the existence of a membranous structure (corbule) at the boundary was overlooked by him. Porter accurately described a "coarsely granular protoplasmic partition," but he also did not recognize the existence of a membrane. Grassi distinguished the corbule ("cestello") in the Italian form, and described it as consisting of numerous rodlets ("bastoncelli"). As described above, the corbule shows no such fibrous or rodded structure in my organisms. The zonal arrangement of thin and dense areas of granules, observed very often in my organisms, does not suggest, to me, the existence of fibres. I conjecture the zonal appearance to be due to thickenings of the substance of the membrane, or a zonal arrangement of the granules, in consequence of contraction of the body or such other causes. Grassi conjectured the membrane to be continuous with the inner layer of the body wall, but I do not think this is so; and I am of the opinion that a layer of the same substance as the membrane may exist under the wall of the bell, keeping the corbule in connexion with the axial core of the nipple. I believe also that the columnar mass hanging from the base of the nipple to the nucleus, and the contents of the axial core, are composed of the same substance. A comparative study of similar structures in allied forms seems to afford us some basis for such conjectures. França described the corbule as an ellipsoidal sac hanging from the base of the nipple. I think his statement may be true, but undoubtedly the thick body wall of the bell, overlooked by him, and the upper half of his "cestello," is nothing but the basal line of the wall of the bell.

According to Kofoid and Swezy (1919) no structure corresponding to the corbule is noticeable in *T. campanula*, and there is a complicated system of fibres and myonemes, called by them the "neuromotor system." They describe the neuromotor system as consisting of oblique fibres, longitudinal ridges with the basal granules of the flagella, longitudinal myonemes, and transverse myonemes; the first three being continuations of the axial core of the nipple ("centroblepharoplast" in their terminology). As far as my experience goes, there are no such fibres or myonemes recognizable in my organisms. As for the corbule in *T. campanula*, I conjecture that a similar but less differentiated structure may be present.

The nucleus is oval (10–18 μ × 8–12 μ), and is provided with a thin but rigid membrane. It consists of a structureless ground substance and a variable

number of chromatin masses, which are found assembled at the centre, forming a large spherical heap surrounded by a clear zone. In the majority of cases, the chromatin masses are distinct in their outline and separated from each other. Sometimes, however, they are not so sharply contoured and distinctly isolated, but loosely connected to form a somewhat reticular mass. The chromatin masses in any one nucleus are of nearly uniform size; but in some individuals they are small in size and numerous, and in others few but large.

Despite my long search, carried out through all seasons, I have been able to find only a small number of dividing individuals. The observations of American authors agree with mine in this point. In the Formosan variety, on the other hand, dividing forms are found not infrequently, and I have had ample material for the study of the process of division, which seems to be identical with that of the variety under consideration. Thus no separate account of the process in the variety *japonica* will be given.

No forms which suggest the occurrence of other modes of development have been observed by myself. Leidy has figured several forms which he thought to be the young, but a contrary view was afterwards put forward by Porter. Some of the forms considered by Leidy to be the young are really met with in *Leucotermes speratus* in association with the present variety. But there is strong evidence indicating that they have nothing to do with *Trichonympha*, as pointed out by Porter, and have to be classified under different genera. The form which was taken by Porter—though with much misgiving—to "present the best evidence of being the young" is not found in *Leucotermes speratus*, though it is frequently met with in *Leucotermes flaviceps* accompanied by the other variety of *Trichonympha*. It is thus quite certain that there is no genetic connexion between Porter's "young form" and the variety now under consideration.

(2) *Trichonympha agilis* var. *formosana* var. nov. (Plate X, figs. 5–12).

This variety is distinguished from the foregoing by its shape, the proportional dimensions of various parts of the body, and by the structure of some parts.

The difference in shape of the two varieties is recognized at a glance (compare Figs. 1 and 5, Plate X). The body length is nearly the same in the two varieties, while the breadth of *formosana* measures only a little more than one-half that of *japonica*. The organism assumes a spindle or a lanceolate shape, in contradistinction to the oval shape of the former variety, and the inclination of the wall of the bell is conspicuously acute. The head (including the nipple and the bell) occupies some one-sixth of the length; and it is thus a little smaller than that of the variety *japonica*. Though the body is slender in this variety, as described above, the nucleus is about the same size as in *japonica*, so that a larger portion of the breadth of the body is occupied by it in this form. Some differences are also recognizable in the nipple and the

wall of the bell. The axial rod of the nipple is devoid of the constriction, behind the anterior knob, seen in the former variety. The inner layer of the wall of the bell is thicker than in *japonica*. The deeply stained zone at the boundary of the inner and the outer layer of the bell is distinct and shows an appearance of a sheet of minute granules closely aggregated. The acuteness of the inclination of the wall of the bell and the proportionally large dimensions of the nucleus are associated with a difference in the shape of the membranous wall of the corbule. In the majority of cases, it assumes the shape of an inverted cone, or sometimes of a cylinder, touching the nucleus tangentially in the vicinity of its equator and stretching behind it.

Dividing forms are occasionally met with in this variety. In the termites taken directly from their nest, or those kept in large vessels under conditions supposed to be natural for them, dividing forms are very seldom met with; and a large number of termites have to be examined before hitting upon any dividing forms. I happened, however, to come across a method of getting dividing forms with less difficulty: namely, by keeping the termites, for several days, in a small glass dish with a small quantity of mud, and a piece of cotton-wool to serve as food. Such conditions acting upon the host seem to induce the multiplication of the intestinal organisms. It is rather remarkable that a large number of dividing forms may be found in a single host, while not a single one is met with in another subjected to the same conditions.

The division processes of the Italian species have been described by Foà (1904), and Kofoid and Swezy (1919) have described those of *T. campanula*. The division processes of the forms under consideration are of the same type as those described by these authors.

Actual division takes place at first in the nipple. It is bisected longitudinally into two equal halves, and a rather thick strand ("fuso esterno" of Foà, and "paradesmose" of Kofoid and Swezy) makes its appearance at the base of the daughter nipples, keeping them in connexion. This strand is somewhat fibrous and is stained rather feebly by iron-haematoxylin. Before the above-mentioned division takes place, nuclear changes usually occur. In the resting stage the nucleus is of the same structure as that of the former variety; but in the variety *formosana* the chromatin masses are more often not distinctly separated from one another. The first indication of the nuclear division is the disappearance of the membranous structure of the corbule, and the scattering of the chromatin masses into the ground substance of the nucleus. There the chromatin masses appear swollen and broken into pieces (Pl. X, fig. 6). The nuclear membrane becomes indistinct and the ground substance becomes denser in its constitution. At about this time, the division of the nipple takes place. The connecting strand continues to be elongated and the daughter nipples are separated by degrees. The nucleus and the strand, which were originally separated by some distance, are now gradually brought nearer to one another until they come to be united as is shown in Pl. X, figs. 7 and 8.

The strand is stretched into a slender filament, gradually attenuating towards the base of the nipple. The nucleus is found lying close by the filament, stretching along its whole length and fixed at both its ends. The chromatin masses become at this stage more or less long thread-like chromosomes, each consisting of several granules loosely arranged in a chain. The nucleus then assumes a spindle shape, lying closely by the filament, and the chromosomes also become arranged in a spindle along its middle portion (Pl. X, fig. 9). They then shorten and assume the shape of rods, provided with several slight constrictions, which exist throughout the rest of the division. The number of chromosomes counted varied from 10 to 14; but in the majority of cases they were found to be 12, which probably represents the actual number. Meanwhile the chromosomes are divided transversely at the equator and the ground substance is also divided at the corresponding place: the halves thus produced then move towards opposite poles along the filament, until they reach a position near the base of each daughter nipple (Figs. 10 and 11). The daughter nuclei are then separated from the filament and come to lie at a short distance behind the bases of the nipples, where the chromosomes break up into chromatin masses and the nucleus returns to its resting condition (Fig. 12). The filament becomes detached from the base of the nipple and soon disappears.

As the nipple is divided, the anterior part of the body becomes very much widened and flattened, so that the whole body assumes the shape of a turnip; the above-described processes taking place under the flattened surface of the anterior part. The flagella are not cast off, nor do they cease to move, and the organism usually continues to display a sluggish motion throughout the whole process of division.

Interesting and important questions are raised by the above-described phenomena of division, and concern the origin of the strand, and the rôle played by the daughter nipples. According to Foà (1904) and Kofoid and Swezy (1919), the strand is directly connected with the bases of the daughter nipples, and no structures such as centrosomes are recognizable at its ends. I have also failed to find any structure such as a centrosome and I am of the opinion that the rôle of division-centre is played by the daughter nipples. Further discussions of these points will be given in Part II.

As in the variety *japonica*, no forms suggesting the occurrence of any other modes of development have been observed in this variety. Forms closely resembling those taken by Porter to "present the best evidence of being the young" of *T. agilis* are met with in company with this form. But they are undoubtedly quite independent of *Trichonympha*, and I refer them to a new genus, *Microspironympha*.

II. *Pseudotrichonympha.*

Hartmann (1910) found three trichonymphids in a species of *Coptotermes*[1] in Brazil, and interpreted them as representing the young, the female, and the male, of a single new species which he called "*Trichonympha hertwigi.*" Soon after the appearance of Hartmann's work, objections to his view were raised by Grassi (1911), and the "sexual forms" of Hartmann were made independent species, each being referred to a new genus, viz. *Holomastigotoides* and *Pseudotrichonympha*, proposed for the "female" and the "male" forms respectively. ("Male" and "female" contrariwise misprinted at that time, and corrected later.) In an article contributed to the *Handbuch der Naturwissenschaften* (Vol. III. 1913), Hartmann subsequently adopted the generic name *Holomastigotoides*, but did not give up his former conception as regards the relationship of the three forms. In *Coptotermes formosanus* three types are also found, and each of these resembles rather closely the corresponding one in the Brazilian termite. The results of my studies of these forms have convinced me that Hartmann's view is by no means right, and not only the "male" and the "female," but also the "young" forms, should be referred to independent genera.

Three more species of *Pseudotrichonympha* have been described recently. In his latest work (1917), Grassi described two new species of this genus, *P. magnipapillosa* and *P. parvipapillosa*, harboured by African (French Guinea) and Australian species of *Schedorhinotermes*. One more species was briefly described by Imms (1919)[2] from the Indian *Archotermopsis*. "*Trichonympha*" *leidyi*, described by Kent (1885), probably belongs to this genus; but his account is not sufficiently detailed to make its precise determination possible. In spite of the resemblances, the species of *Pseudotrichonympha* in our termites shows some differences from the Brazilian, Australian, African, and Indian species, so that it should be regarded as new, and I have therefore proposed the name *Pseudotrichonympha grassii* for it. A discussion of Hartmann's view of the relations between the three types will be given later.

Pseudotrichonympha grassii sp. nov. (Plate XI, figs. 14–23).

This is one of the largest of the protozoa found in our termites, reaching a size visible by the naked eye. The body usually assumes a spindle shape, measuring 200–300 μ in length and 50–120 μ in breadth. Sometimes it assumes a slender lanceolate shape, and its length exceeds 500 μ in extreme cases. It is thus shorter and broader than Hartmann's species and those described by Grassi. It is markedly variable in shape as compared with *Trichonympha* and other forms found associated in *Coptotermes*.

In general organization it resembles *Trichonympha*, and the body may be distinguished into an anterior region or head, and a posterior region or body.

[1] Incorrectly called *Captotermes* by Hartmann.

[2] Incorrectly called "*Trichonympha* (*Holomastigotoides*)" by him.

The anterior region occupies only a small portion as compared with that of *Trichonympha*, and measures 30–40 μ in height, so that the posterior region is from six to nine times as long as the anterior. The anterior region may again be divided into a nipple and a bell, as in *Trichonympha*, though no circular fissure ("citartrosi" of Grassi) separating these parts is to be found in this species of *Pseudotrichonympha*. The bell occupies some two-thirds of the anterior region, measuring some 20 μ in height and 40–50 μ in transverse diameter at its margin.

The nipple is of similar construction to that of *Trichonympha*, but differs in several details (Pl. XI, fig. 15). The axis of the nipple is not formed of a simple column, but consists of a tubule with a round ball situated at its anterior end. The layer surrounding the axis is thickly traversed by flagella and resembles the outer layer of *Trichonympha*, no layer corresponding to the inner layer being recognizable around the axis. The wall of the bell consists of two layers, just as in *Trichonympha*, the outer one being continuous with that of the nipple. The inner layer ends anteriorly at the edge of the base of the axis. It measures about 2 μ in thickness in the middle and becomes gradually thinner towards the ends. The tubular column is slightly thickened at both ends, the posterior end being the thicker. Its wall is fairly thin, and becomes also gradually thicker towards both ends, especially towards the posterior. The ball is solid, and its diameter is nearly equal to that of the anterior end of the tubular column. It fits closely into the opening of the latter; that is, only a hemisphere of the ball appears at the top of the axis, and the opening of the tubular column is perfectly closed. Both the wall of the tubule and its contents are transparent, homogeneous, and structureless, no granules as seen in *Trichonympha* being recognizable in the contents of the axis of this organism. The wall and the contents, moreover, are of nearly the same refractive index, so that these two constituents of the axial column are not easily distinguishable. The ball is usually indistinctly recognizable in fresh specimens, perhaps owing to its having nearly the same refractive index as the contents of the cap.

The layer around the axis is of the same structure as the outer layer of *Trichonympha*, but its ground substance is not of uniform density in this organism. It is dense immediately around the column and has the same appearance as the outer layer of *Trichonympha*, in both fresh and stained preparations. The peripheral half is, however, much thinner. The boundary between these two layers is more or less distinctly discernible in some individuals. The flagella are shortest at the anterior and longest at the posterior region of the head, the free portions of them measuring 6–8 μ and 25–30 μ in these two regions respectively. They are more markedly inclined backwards than in *Trichonympha*, and the cap is much taller, its edge being found near the middle of the nipple. In living specimens the cap can be seen to undergo changes of shape in this species, but it is rather rare to see it assuming the shape of a hemispherical bowl.

In fixed preparations the ball is very feebly stained by iron-haematoxylin, but the wall of the tubular column is stained deeply, while its contents stain very feebly or not at all. The outer surface of the tubular column is stained intensely, and a granular appearance is here recognizable in some specimens. The boundary of the outer and the inner layers in the bell is also stained deeply, and shows a granular appearance. The inner layer appears quite the same as the corresponding layer in *Trichonympha*, in fresh specimens as well as in stained preparations. It cannot be doubted that the flagella of the nipple arise from the above-mentioned deeply stainable zone lying immediately on the surface of the axis, and those of the bell from the similar zone at the base of the outer layer.

It may be added here that the descriptions of the anterior region in the species studied by Grassi and Hartmann do not agree with mine. According to Hartmann, in the Brazilian species, the column and the body at the top are both solid, and the latter is not spherical but hemispherical, lying on the flat surface of the anterior end of the column. In the African and the Australian species, Grassi describes both structures as hollow, filled with liquid. As regards the layers surrounding the axial core in the nipple and those in the wall of the bell, moreover, the descriptions of these species do not agree with mine. According to Hartmann, there is no layer corresponding to the inner layer, both in the nipple and in the bell. That is to say, the axis of the nipple of "*Trichonympha hertwigi*" is directly surrounded by a layer corresponding to the outer layer of *Trichonympha*, just as in our species, but the wall of the bell consists also of a single layer, thus differing from our species. Grassi, on the other hand, distinguishes two layers, both in the nipple and in the bell, and he believes that the flagella arise at the base of the inner layer. I am not quite certain whether or not such differences really exist between these species, but I doubt the accuracy of the authors' observations as regards some of the above details.

The entire posterior region of the body is covered by fine flagella densely distributed, excepting only over a small portion at the posterior extremity. They are of almost uniform length over the whole surface, measuring 16–20 μ. They are arranged in numerous rows, giving the body an appearance as if it were beautifully striped. Forming the body wall there is one distinct layer, measuring about 2 μ in thickness. It exists from the edge of the bell to the posterior end, but becomes extremely thin over the small bare area at the hinder extremity. The layer is of the same structure as the basal part of the outer layer of the bell, and at the edge of the bell it can be clearly made out that the two are continuous. The basal granules of the flagella are localized at the base of the layer, where a distinct zone of other minute granules, deeply stainable with iron-haematoxylin, is recognizable. The basal portions of the flagella, being embedded in rows in this layer, appear to form a kind of band or plate. The rows of flagella are arranged spirally, uniformly separated by some 2 μ in the middle region, but becoming gradually closer towards the

margin of the bell and the posterior end. The winding of the spiral rows is left-handed (laeotropic)[1]. The outer surface of the body wall seems to be destitute of any specially differentiated membrane.

The endoplasm appears minutely granular and no alveolar structure is recognizable. Beneath the body wall, a zone of coarse and deeply stainable granules, as seen in *Trichonympha*, is recognizable. A thin but distinct zone of clear and structureless substance between the basal layer of the body wall and this layer of granules is also present in both the anterior and posterior regions.

The organism seems to be voracious, and contains a greater or less abundance of food débris, consisting chiefly of wood fragments. The food bodies are commonly limited to the portion posterior to the nucleus, but they may be found very rarely in the region between the nucleus and the anterior end.

The nucleus is found usually a little anterior to the middle of the body, though sometimes situated far posteriorly, and there is no structure, such as a corbule, connecting it with the body wall. It is round in shape, measuring 16–20 μ in diameter. Its wall is peculiar in this form, appearing very thick and abnormally tough. In fresh specimens the wall is markedly refractive, and the internal structure is not visible. In the majority of cases the contour is not even, and a wide groove or distinct folds may be commonly found running over the surface (Pl. XI, fig. 14). This peculiarity of the nuclear wall can be seen clearly in living specimens. The inner structure of the nucleus differs somewhat from that described by Hartmann and Grassi. It consists of a network of chromatic threads and clear ground substance. In some individuals the network is loose, while in others it is rather dense, presenting a spongy appearance. It is a peculiarity of this species that the network forms a more or less smooth and distinctly contoured mass, leaving a space under the nuclear wall (Pl. XI, figs. 14 and 17).

Dividing forms are frequently met with in this species. The process of nuclear division is peculiar, and different from that of the preceding genus. At the first stage in division, a peculiar structure makes its appearance at the anterior region, as is shown in Pl. XI, fig. 18. It is a thread hanging in the endoplasm, bent at several points, and provided with a small sphere at its free end. The opposite end of the thread could not be made out, because of the feebly stainable character of the thread and owing to the dense zone of granules distributed under the wall of the bell. However, it cannot be doubted from its behaviour that it is connected with the base of the axis of the nipple. The sphere and the thread stain more or less faintly with iron-haematoxylin. The sphere probably approaches the nucleus rapidly, until it comes to lie on its surface, and the thread then appears as a straight or slightly wavy line.

[1] The direction of the spiral rows of flagella, in all of the other accompanying forms found in the intestine of *Coptotermes formosanus*, is just the reverse of that in this form, namely right-handed (dexiotropic). In Hartmann's figure of his "male" form the rows are drawn as left-handed spirals, while those of his "female" forms (*Holomastigotoides*) are shown partly as left-handed and partly as right-handed.

Division takes place when the sphere and the nucleus are brought together, the sphere, the thread and the nipple almost simultaneously dividing into two. The axial column of the nipple splits longitudinally into two halves, the split beginning at the posterior end and extending forwards. The surrounding layer also divides, each half being accompanied by one of the daughter axes. The ball at the top of the column is not divided completely at this stage, but is constricted first into a dumb-bell, and remains connecting the top of the daughter axes until just prior to the completion of the whole process. When the little sphere at the end of the thread divides, the two daughter spheres are situated on the surface of the nucleus, each connected with the base of the daughter nipple by means of a daughter thread, formed apparently by a splitting of the original single thread. The most remarkable feature observable at this stage is a thick and distinct strand connecting the daughter spheres, on the surface of the nucleus (Pl. XI, fig. 19). It is identical in its structure with the strand seen in *Trichonympha*, though it is, in *Pseudotrichonympha*, more conspicuous and distinctly fibrous. The above processes must take place very quickly, since I have not yet seen a specimen with the nipple already divided but the strand not yet found on the nucleus. Owing to its fineness and its faint stainability, the thread may frequently be invisible, but the strand never escapes the observer's eye. In this respect, Hartmann's description appears somewhat singular to me. He described some individuals with divided nipples, but in none of these forms does he seem to have noticed either the strand or the thread connecting the nucleus with the nipple. It is certain, however, that only a few forms in an early stage of division were observed by Hartmann, and he did not follow the subsequent processes. Thus it is not clear whether a strand makes its appearance in the Brazilian species later than in our species, or whether it does not appear at all.

The strand is now gradually elongated. The nuclear membrane persists throughout the whole division process, and the spheres are always attached firmly to it. Consequently elongation of the strand is accompanied by an alteration in the shape of the nucleus. The strand lies transversely and the nucleus is disposed, in the majority of cases, along the whole of its length, and appears as a segment of a circle (Pl. XI, fig. 20). Hand in hand with the alteration of the shape of the nucleus, changes occur also in the chromatic network. Chromosomes are formed, by the chromatic substance forming an irregular network or a spongy mass, and coming gradually to assume the form of more or less distinct threads with nodular thickenings. These chromatic threads become gradually distinct and uniform in thickness, coiled in the nucleus, appearing like the spireme in metazoan cells. Finally they resolve themselves into a large number of chromosomes, arranged along the strand and fixed to the spheres at both ends. They then split transversely into pairs of daughter chromosomes. I think it probable that the chromosomes do not split simultaneously, but are divided one by one. They are of nearly uniform thickness, but their surface is rough. The daughter chromosomes fixed to the polar

spheres differ remarkably from each other in their length; and they are very difficult to count, so that I cannot give a definite figure for their normal number. The difficulty is chiefly due to the fact that some of the chromosomes, where they are fixed to the sphere, are bent at one or more points; and moreover, some of them are coiled around each other. The number seems to be more than 12.

While the above processes are going on, both poles of the nucleus become gradually thickened and rounded. At this stage the chromosome-formation is completed, and a constriction appears at the middle of the nucleus, indicating the plane of division (Pl. XI, fig. 23). The strand becomes still more elongated until it reaches twice or thrice the length of the diameter of the resting nucleus. The just divided daughter nuclei are then found attached to its two ends (Pl. XI, fig. 21). Finally the strand is broken and the nuclei are detached from it, leaving the spheres behind, hanging from the nipples by means of their threads, with the broken remains of the strand still attached. The mode of breaking of the strand is noteworthy. The elongated strand is usually seen to be conspicuously swollen in the middle, and it is on either side of this swollen portion that the break occurs. Thus the strand is broken into three, and its fibrous structure is beautifully demonstrated at the fractured places. It is at this stage that the constricted ball on the axial column of the nipple is severed, and the division of the nipple is thus completed. The division of the nipple is quickly followed by the division of the bell. Complete division of the body seems to be effected rather slowly, and individuals with a divided anterior portion, such as that shown in Pl. XI, fig. 22, are often observed in fresh preparations, remaining undivided for some time. In the young animalcules soon after division, remnants of the strand and the thread may be seen. Unfortunately I could not ascertain the fate of these structures. Reorganization of the normal structure in the young seems to be carried on quickly.

III. *Teratonympha.*

An organism with a remarkable structure is found in the *Leucotermes* of both Japan proper and Formosa, and for it I propose the new generic name *Teratonympha*[1]. It shows so many peculiarities in its organization that it seems at first sight to differ greatly from all the hitherto described protozoa of termites. However, when the organization of the head is carefully studied, a fairly close relationship can be clearly made out between this form and the preceding genera; and the arrangement of its flagella can be homologized with that seen in Holomastigotidae.

The individuals in *Leucotermes speratus* of Japan proper are somewhat different from those in *L. flaviceps* of Formosa, and the rather slight but distinct differences between them can be recognized constantly; so I have

[1] In my original paper in Japanese (1917) I wrote this name as "*Teranympha*"—an orthographic error which I now correct.

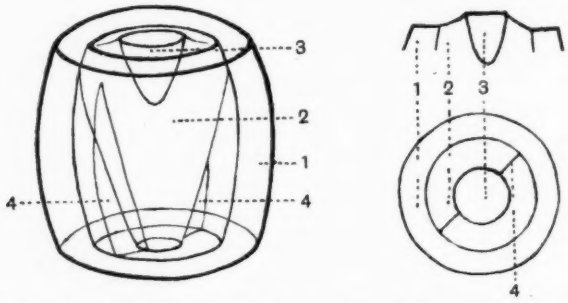
distinguished them as *Teratonympha mirabilis* (type species) and its variety *formosana*, respectively.

(1) *Teratonympha mirabilis* gen. nov. et sp. nov. (Plate XII, figs. 26–31, and Text-figs. A and B).

This is one of the largest of the protozoa in Japanese termites and much the largest of the forms found in *Leucotermes*; and its large size, coupled with its monstrous appearance, at once attracts the observer's attention. Ordinarily the organism assumes the shape of a club, being rounded at the anterior end and somewhat pointed posteriorly. The differentiation of the parts of the body of this organism is far more remarkable than in *Trichonympha* and *Pseudotrichonympha*. The anterior portion of the body has a remarkably complicated construction, and the posterior region appears very peculiar, being apparently segmented like a cestode. The specially differentiated anterior part, or head, has the shape of a concavo-convex lens. The body is widest near the head and gradually decreases in diameter towards the posterior end. The organism usually measures 200–250 μ, but sometimes 300 μ or more, in length, and 40–50 μ in width at the widest portion. In the posterior region the body wall is not smooth, but is provided with numerous transverse and more or less prominent and sharp ridges, which are inclined backwards. The regular metameric arrangement of these ridges gives the body the segmented appearance which suggests the proglottids of a tape-worm. The appearance of the endoplasm also tempts one to think that the body is made up of successive segments, as in the cestodes. But careful observations reveal that the differentiation is limited to the body wall, and no septa are to be found crossing the endoplasm. The number of the ridges varies from 18 to 30, or sometimes even more, in proportion to the length of the body. As the animalcules are subjected to unfavourable conditions, their shape changes more or less quickly to that of a spindle or turnip, until finally they become rounded and burst. The flagella are very conspicuous in this form. Very long ones are found closely distributed at the head, as in the preceding genera. On the surface of the body, however, they are not distributed diffusely, but are arranged in parallel rows, each of the transverse ridges being provided with one row of them.

The head consists of two parts; namely, a thick axial column or cylinder and a peripheral layer traversed by numerous flagella, arising at the surface of the column. The peripheral layer encircles the axial column from top to base, leaving the frontal surface uncovered, and becomes gradually thinner towards the sides as it curves backwards. The axial column itself is ordinarily barrel-shaped, being slightly narrowed towards the top and the base. It measures some 10 μ in breadth and 15 μ in height. Its shape is, however, variable to a certain extent, and it is often narrowed at the anterior end only. Its complicated organization is shown in Text-fig. A. As it is quite transparent, the details of its structure can be made out in the living organism.

Differential staining of the several parts is not easy, and stained preparations are not so suitable for studying the structure of the head. The principal parts of the axial column are a circumferential wall or rind of a tubular form, and a central cone, or core, fixed within it. The tubular rind is of a nearly uniform thickness, measuring 1·5–2 μ; and circular openings, measuring 4–5 μ in diameter, are found at both its ends. The conical core is widest at the anterior end, fitting into the upper opening of the tubular rind and gradually decreasing in thickness posteriorly so that it resembles an inverted cone, having the greater part of its surface separated from the tubular rind. The conical core itself is not composed of a single substance, but a body assuming the shape of an inverted cone or hemi-ellipsoid is found embedded in its anterior or basal portion. The main portion of the core completely covers this smaller conical body, leaving only the flat surface of the latter, corresponding to the basal plane of the cone, uncovered. The front end, or base, of the smaller conical body, and the tubular rind together form a smooth anterior



Text-fig. A. Diagrammatic representation of the axial core at the anterior end of the body of *Teratonympha*. 1, tubular rind; 2, outer cone; 3, inner cone; 4, vertical partition.

surface for the whole structure; but the central cone is a little more advanced in position than its surrounding tube, so that the frontal surface of the main part of the axis slopes gradually from the junction of the two cones towards the boundary between the external cone and the tubular rind. The interval between the core and the rind is more or less spacious, and is filled by a structureless protoplasmic substance of dense consistency. Though the rind and the greater portion of the conical core seem completely separated from each other, careful observations show that there is a distinct vertical partition on both sides, stretching from the posterior end of the whole axis to the point where the tubular rind and the conical core are in contact anteriorly. The two partitions are so situated as to lie in one plane, or nearly so, and divide the space between the apex of the cone and the tube into two nearly equal halves.

The tubular rind takes iron-haematoxylin very feebly or not at all, excepting a zone of limited breadth at the inner surface, which is stained rather

more deeply, just as in the inner layer of the nipple and the bell of *Trichonympha*. The outer surface takes dyes intensely, and shows an appearance of an aggregation of minute and very deeply stained granules, indicating that the flagella arise therefrom. Thus it seems probable that the rind is the homologue of the inner layer of the head of *Trichonympha* and *Pseudotrichonympha*. The conical core and the contents of the space around it stain more or less deeply. The outer surface of the cone, and the vertical partition connecting it with the inner wall of the tubular rind also stain deeply, but no granular appearance is there visible. Differential staining of the inner and outer cones of the core at their apices is not easy, but the outer seems to stain a little more faintly.

The peripheral part of the head is identical in structure with the outer layer in the anterior region of *Trichonympha*. It is closely traversed by flagella, which arise at the junction with the central axis. Owing to the flattening of the head, the proximal ends of the flagella embedded in the peripheral layer are very long, so that their distal or free ends are nearly equal to the embedded portions in length. The flagella are shortest anteriorly, and gradually increase in length posteriorly: the longest ones measure 20–25 μ, including the embedded portion. Behind the head, a thin but distinct layer is noticed lining its concave surface. The flagella do not arise from this layer, but run parallel to it.

The anterior surface of the whole axis is apparently naked, and provided with no special structure, such as the cap on the nipple in the preceding genera. Careful observation, however, shows that there is a thin membrane lying on the frontal surface of the head—this membrane being hardly noticeable, owing to the absence of any space between it and the frontal surface.

In vigorous animalcules the head is either swung actively from side to side, or is held straight and the axial column alone displaced backwards and forwards along the long axis of the body. In the former case, the shape of the head becomes markedly asymmetrical; in the latter, the central portion of the head is alternately drawn in and pushed out in rapid succession.

From the above descriptions, it will be recognized that the head of this form is homologous in organization with the anterior region of *Trichonympha* and *Pseudotrichonympha*. As already indicated, the peripheral part traversed by the flagella, and the tubular rind of the axial column, are respectively comparable with the outer and the inner layers of the anterior part of *Trichonympha*. It seems probable also that the conical core and the inner conical body, at its anterior or basal end, are homologous respectively with the columnar axis, and the ball at its anterior end, in the nipple of *Pseudotrichonympha*. As regards the contents of the space between the outer cone and the tubular rind, comparative studies of allied forms afford strong evidence that it is homologous with the contents of the axis of the nipple in *Trichonympha*. A discussion of the nature of these structures will be given in detail in Part II.

A single large nucleus is situated at a short distance behind the axial column. It appears usually oval in shape, measuring 10 μ or rather more in diameter. It is not suspended freely in the endoplasm, but is enveloped in a sac, the nuclear sac, hanging from the base of the axial column of the head. The sac has the shape of a flask with an ovoidal or globular body and a slender neck. It is, moreover, provided with a flange-like extension in the horizontal plane at the equator of its globular portion. The nucleus almost fills the main body of the sac, which fits itself very closely round the nuclear membrane; and consequently the sac is usually inconspicuous on the surface of the nucleus, which seems to be united to the base of the axial column by means of a membranous tube at its anterior pole, and provided with a horizontal extension at its equator. This horizontal flange or plate is distinct in the vicinity of the nucleus and becomes gradually indistinct towards the body wall, so that its entire course can be clearly followed only in good preparations. The tubular neck of the sac varies somewhat in length, according to the condition of the head and the size of the nucleus. During the movements of the animalcule, the shape of the nucleus is also frequently modified by the pressure exerted by the sac, especially when the nucleus itself is large. The nuclear sac is continuous at its anterior end with the substance filling the space between the rind and the core of the axial column of the head. I am of the opinion that the sac is homologous with the corbule of *Trichonympha*, which keeps the nucleus in connexion with the body wall.

The nucleus is of the same type as in *Trichonympha*. No trace of linin reticulum is to be seen, and fairly large chromatic masses are found assembled at the middle portion, leaving a wide zone of clear ground substance peripherally. In some forms the chromatic bodies are distinct in outline, as is common in *Trichonympha*; while in some others they are united to form a rough network, as in *Pseudotrichonympha*. A single nucleolus is clearly discernible in nearly all individuals, situated near the centre of the nucleus and surrounded by a clear space.

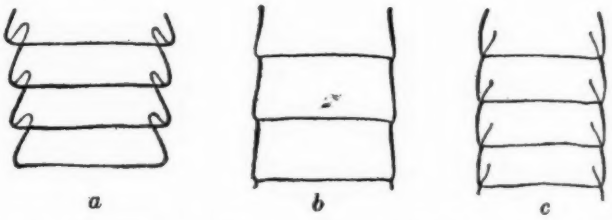
As already noted, the body is provided with numerous transverse ridges on its wall. In the normal state each of the ridges has the shape of a drawn out and flattened fold, inclined backwards so that a distinct groove is formed under it (Text-fig. B, *a*). The ridges, however, are not necessarily distinct in every individual, and when the body is abnormally elongated they almost disappear (Text-fig. B, *b*). In some cases, on the other hand, they are pressed so closely against the body that the grooves entirely disappear, and the surface then appears almost smooth (Text-fig. B, *c*).

In the endoplasm two portions are distinguishable. The main portion is similar to that of *Trichonympha* or *Pseudotrichonympha*, being more or less coarsely granular and appearing grey in colour in the living animal. The ridges, on the other hand, consist of a transparent and structureless protoplasm of dense consistency.

The body wall differs from that of the preceding forms, and the mode of

arrangement of the flagella is also peculiar. There is no special layer, as seen in the preceding forms, on the body wall, but it is provided with a thin but rigid membrane or periplast. The flagella are not distributed diffusely, but are arranged in distinct transverse rows, and become free at the edge of each of the transverse folds. A band of deeply stainable granules is distinctly discernible at the bottom of each groove formed between two successive ridges. It is from this band that the flagella arise, and it can hardly be doubted that a row of basal granules exists somewhere in the band, though its precise location is not clearly visible. The flagella of this form differ from ordinary flagella or cilia in their relation to the body wall, their basal portions being adherent to the periplast. They run backwards, fixed on the surface, until they reach the summit of the fold, where they become free. Each flagellum measures about 30 μ in length, the free portion constituting about two-thirds of the entire length (Pl. XIII, fig. 53 *a*).

In the anterior part of the body, some peculiar filamentous structures, varying in size as well as in number, are usually seen in the endoplasm. They



Text-fig. B. Diagrammatic drawing of various conditions of the ridges on the body wall of *Teratonympha*. Explanation in the text.

are commonly bent at the anterior end, the main portion of the filament usually lying longitudinally. They are composed of a transparent substance showing sometimes a fibrous structure, and are covered thickly by minute but deeply stainable granules. From two to five may be present, and they are invariably found close behind the nucleus (cf. Pl. XII, fig. 24).

Dividing forms are occasionally met with in this species as well as in the Formosan variety. The process of division is analogous in essential points to that of *Trichonympha* and *Pseudotrichonympha*, but is more complicated. In individuals beginning to divide, the body is shortened and widened, especially at the anterior end, so that the organisms become turnip-shaped. The axial column of the head is divided longitudinally. Pl. XII, fig. 28 represents an individual in an early stage of the process: the axial column is already split into two equal halves, and a filamentous strand, identical with that seen in *Trichonympha*, is seen lying near its base. The two ends of the strand of this form are separated from the column; but there is a thread-like structure connecting each end of the strand with the base of each daughter column (Pl. XII, fig. 29), like that seen in *Pseudotrichonympha*. At either end the strand is provided with

a minute sphere of dense protoplasm containing a deeply stained granule. The formation of this strand seems to occur very quickly, for specimens at this stage are extremely rare. In Pl. XII, fig. 27 is depicted an individual in an earlier stage of division: there is a horseshoe-shaped structure stained moderately by iron-haematoxylin, with a globule, stained deeply, at each end, and a thread connecting it with the base of the recently divided axial column. The horseshoe-shaped body is probably an early stage in the development of the straight strand.

As in *Trichonympha*, indications of division are recognizable in the nucleus earlier than in the head. The first sign of division seen in the nucleus is the disappearance of the nuclear sac. In the nucleus, now free in the endoplasm, the chromatic bodies are resolved into fragments, the ground substance becomes gradually denser, and the nuclear membrane becomes indistinguishable (Pl. XII, fig. 28). While the above processes are going on in the nucleus, the axial column is divided, and the strand makes its appearance and continues to grow thicker and longer. The nucleus now gradually draws near the strand, coming to lie closely under it, and finally becomes united to it, as in *Trichonympha*.

At this stage chromosome formation is completed. The chromosomes are arranged rather regularly side by side. The mode of chromosome formation seems analogous to that of *Trichonympha*. The chromatic granules are so arranged as to form a certain number of chain-like threads. In some individuals several pairs of chromatic threads are observed lying closely parallel to each other (Pl. XII, fig. 27), and I am inclined to take this appearance to indicate that the daughter chromosomes are formed by longitudinal splitting. The fact that the chromosomes when arranged in a single plane during later stages, such as that shown in Pl. XII, fig. 29, are equal in length to those in the daughter nuclei, seems to support the above supposition, and show us that the splitting of the chromosomes occurs early in this species. In this species, the number of chromosomes counted varied from 20 to 30. I have not been able to determine their number exactly. They are not uniform in thickness but nodular, and do not differ distinctly from each other in their length.

As the strand and the nucleus are brought into connexion, the daughter chromosomes move towards the opposite poles, and the division of the ground substance then follows. The fate of the strand in this organism is different from that of the similar structure observed in *Pseudotrichonympha*. It does not remain long attached to the daughter nuclei, but becomes detached from them, and does not break into three as in *Pseudotrichonympha*. In Pl. XII, fig. 30 an individual (only two-thirds of the body depicted) in a late stage of division is shown. The strand is here seen detached from the nuclei, and one of the latter is seen still connected with the daughter column by means of its filament. In each daughter nucleus, now just separated, we see a large spherical mass of dense protoplasm, with a granule near one of its poles: the chromosomes are radially arranged, fixed to the spherical body at the side opposite

to that where the granule is situated, and embedded in the ground substance, which now has a distinctly oval shape (Pl. XII, fig. 30). The thread connecting the daughter nucleus with the daughter nipple soon disappears, and reorganization of the nuclear structure and the reformation of the nuclear sac are carried on. In Pl. XII, fig. 31, is shown a young nucleus undergoing reorganization. Here we see the ground substance of the nucleus becoming reniform, with the chromatic threads embedded in it, and a distinct clear zone surrounding the whole mass. The latter structure is peculiar to this form. The boundary between the clear zone and the endoplasm is distinct, indicating clearly the existence of a membrane, and one deeply stainable granule is found on its anterior border. As regards the nature of the above-mentioned structures, I believe that the reniform mass represents the nucleus in process of reorganization, and the membrane at the outer boundary represents the new nuclear sac. Unfortunately I could not completely trace the formation of the new nuclear sac; but I believe that the neck and flange-like part are developed from the above-mentioned membranous wall, and hand in hand with the process of reorganization, the clear zone under the membrane becomes reduced, until it finally almost disappears around the nucleus. Some further discussions of these points will be given in Part II.

(2) *Teratonympha mirabilis* var. *formosana* var. nov.
(Plate XII, figs. 24 and 25).

As already remarked, the form of this genus found in the termites of Formosa differs from that just described, occurring in Japan proper, and was regarded as a distinct variety. The variety *formosana* is distinguishable from the type species by the following characters (see Pl. XII, fig. 25). The neck of the nuclear sac is shorter, being usually half as long. Further, the main body of the sac enveloping the nucleus is found in the preceding form applied closely to the nuclear membrane, so that the sac is not usually clearly distinguishable from the nuclear wall; but in this variety it is separated from the nuclear membrane, leaving a distinct space between them. Both these differences are clearly visible in the living animals, as well as in stained preparations.

Dividing forms are rather frequently met with in this variety, and the process of division is identical with that observed in the preceding form.

IV. *Microspironympha.*

This is a new genus proposed for a small form harboured by the Formosan species of *Leucotermes*. Some of the forms taken by Leidy (1881) and Porter (1897) to represent possible young stages of *Trichonympha agilis* resemble this form closely, but owing to the insufficiency of the descriptions of the American authors, the identification of their forms is hardly possible. As compared with *Trichonympha*, our form is remarkably simple in organization, and differs so distinctly that the existence of a genetic connexion is hardly

imaginable. Their independence cannot be doubted also from the fact that the present form is found accompanied by *Trichonympha* in the Formosan termites only: in the termites of Japan proper, *Trichonympha* does not accompany any form of this type. It is possible that some of the forms described by Hartmann (1910) as young stages of *Trichonympha* also belong to this genus, or to *Spirotrichonympha* of Grassi; but from Hartmann's description and figures of the anterior end of his organisms, it is impossible to identify them with certainty. In my former paper (1917) in Japanese, I referred this organism to the new genus *Spironympha*. Grassi (1911), however, had previously proposed *Spirotrichonympha* for a different form; and therefore, as my name was merely an abbreviation of the same name, in order to avoid confusion I now propose to call the present form *Microspironympha*.

Microspironympha porteri gen. nov. et spec. nov. (Plate XIII, figs. 32–38).

This form is somewhat variable in shape, but commonly fusiform, the length being about twice the breadth. The anterior half of the body is provided with a fairly rigid periplast, and displays little alteration of shape, being usually in the form of a tall cone. The posterior half, on the other hand, is variable in shape. It is rounded in some forms (Pl. XIII, fig. 33), while in others it is prolonged and pointed at the end, giving a spindle-like appearance to the body. In some forms, the posterior end is drawn out into a slender tail-like appendage, variable both in shape and in size. When carefully studied, a slender rod, resembling the axostyle of some species of trichomonad flagellates, is recognizable running the entire length of the tail-like portion (Pl. XIII, fig. 34). The rod is transparent, slightly refractive, and not stained with any dyes. It is only in the slender portion that it is clearly recognizable, and it is never visible in individuals with rounded posterior ends. It is probable, however, that it exists in the majority of individuals, but is invisible in plump forms, being hidden in the endoplasm. Though the anterior end of the rod cannot be made out in any individuals, it may be safely concluded from its behaviour that it is of limited length and is not connected firmly with any part of the body at its anterior end. In this respect it is less developed than the axostyle of trichomonads, and neither serves as a skeleton for the whole body, nor for the control of its movements as in these flagellates.

The organism is small in size for a trichonymphid, spindle-shaped individuals measuring 20–55 μ in length and 10–30 μ in breadth, piriform ones 20–40 μ in length and 18–30 μ in breadth (the tail-like portion being excluded).

Under the periplast of the anterior portion, several narrow bands, consisting of minute granules deeply stainable with iron-haematoxylin, are noticed running in parallel spiral lines. The number of these narrow bands appearing in side view varies commonly from 6 to 8. Careful study shows that there are really four of these bands starting at the anterior tip of the body and turning round spirally towards the posterior portion (Pl. XIII, fig. 35). The direction of the spiral of the bands is always dexiotropic, as in the forms

described by Porter. The bands do not reach the posterior end, the hinder portion of the body being free from them. In some individuals, measuring some 20 μ in length, the anterior region provided with the bands is nearly equal to or rather larger than the posterior region which is destitute of them. As the animalcule grows larger, the bandless posterior portion is chiefly increased in size, so that in large individuals the anterior region is commonly but little larger than in small forms, while the posterior region is relatively very voluminous, its length measuring two, three, or more times that of the anterior region.

It is from these spiral bands that the flagella arise, the basal granules of the flagella being situated in them. The flagella are rather conspicuous in this organism. They are shortest anteriorly and increase in length towards the posterior, the hindmost being some three times as long as the shortest at the anterior end. In large forms, measuring 30–40 μ in length, the shortest flagella measure some 10 μ and the longest ones some 30 μ, so that a large number of them pass over the hinder part of the body. In small forms the flagella are proportionally somewhat longer. The shortest flagella situated anteriorly are directed forwards and are swung vigorously. The longest ones, on the other hand, are directed backwards: they lie more or less closely on the surface of the body, and do not move so actively.

The endoplasm seems less granular than in most other trichonymphids. It is remarkably viscid and a large amount of food débris is commonly found in the posterior region. The most remarkable character of this form is that it seems omnivorous, and not only wood fibres but other protozoa are found in the endoplasm, probably ingested as food. It is very common to see individuals with their endoplasm gorged with organisms such as spirochaetes, small trichonymphids, or other flagellates, the shape of the body being sometimes remarkably altered owing to the large size of the ingested organisms.

The nucleus is situated near the anterior end. It is oval or round, measuring 4–5 μ, or sometimes as much as 7 μ in diameter. It contains usually a pair of chromatic bodies, hanging from the anterior surface and suspended in the homogeneous ground substance (Pl. XIII, figs. 32–34). In the majority of cases, these chromatic bodies are club-shaped, and are independent of one another, hanging side by side. In some forms, however, their posterior ends or the greater part of them are fused together, so as to form, in extreme cases, a body of an oval shape, attached to the anterior wall of the nucleus by means of two mammillary projections. The chromatic bodies usually stain very deeply and almost homogeneously, though in good preparations a more faintly stained ground substance with intensely stained granules embedded in it can be distinguished. The nucleus is surrounded by a dense and structureless substance and is very distinct in its contour, though the membrane is not clearly recognizable.

The nucleus is not suspended freely in the endoplasm, but is connected with the anterior tip of the body by means of a special structure. This is a

distinct tubular organ of uniform thickness, measuring some 1 μ in breadth and 4–7 μ in length. It is clearly visible in living specimens as a transparent and refractive rod. It takes iron-haematoxylin intensely, its wall appearing as though consisting of closely packed granules. In the majority of cases, its posterior end is directly connected with the nuclear wall, and it is at their point of contact that the chromatic bodies are attached. In many cases distinct granules can be recognized at the two points of connexion of the chromatic bodies and the nuclear wall. The tubular structure is neither closed nor provided with a special structure at the anterior end; and it is from the edge of its circular anterior opening that the characteristic bands of deeply stainable granules lying under the periplast originate (Pl. XIII, fig. 35). The substance forming the contents of the tubule is dense and homogeneous, and takes eosin somewhat intensely. As described above, the nucleus is surrounded by a layer of dense protoplasm, and this layer is especially conspicuous in the front part of the nucleus; that is, round the posterior part of the tubule. In large-sized forms the tubule is frequently detached from the nucleus, or its hinder portion is indistinct, and it is apparently kept in connexion with the nucleus by means of the particularly dense surrounding mass of protoplasm (Pl. XIII, figs. 36 and 37).

Dividing forms are met with very rarely in this species, and despite my eager search, only a few forms in later stages of the process were observed. In Pl. XIII, fig. 38, is shown an individual in a stage just before the completion of division. There we see a filamentous structure, its central portion forming a distinct filament, and its ends consisting of deeply stained granules arranged in a chain. Two distinct chromosomes are found symmetrically situated near the ends of the filamentous portion, embedded in a mass of homogeneous protoplasm. Unfortunately I have been unable to find sufficient material to ascertain the origin of these structures. It cannot be doubted, however, that the process of division is analogous in essential points to that of *Trichonympha*. Further discussion of these points will be given in Part II.

V. *Holomastigotoides.*

This is the genus proposed by Grassi (1911) for an organism found in the Brazilian species of *Coptotermes*, and formerly distinguished by Hartmann (1910) as the "female" or "Form B" of "*Trichonympha hertwigi*." As already remarked, there are sufficient reasons to disprove Hartmann's conception regarding the genetic relationship between his "male" and "female" forms, and Grassi's action in establishing the genus was undoubtedly right. As regards the affinity of his "female form" with other forms, Hartmann remarked: "sollte sie sich bei weiteren Untersuchungen wider Erwarten doch als eine besondere Art herausstellen, so müsste sie wohl in der Gattung *Dinenympha* gestellt werden." *Dinenympha*, however, represents a totally different type, provided with four or eight peculiar flagellar cords and differing also in other important characters.

As noted already, forms presenting a type of organization closely resembling that of Hartmann's "female form" are also found in *Coptotermes formosanus*, in company with the forms representing the type of his "male form," or *Pseudotrichonympha*.

Three species of *Holomastigotoides* are distinguished by Grassi (1917)—*H. hertwigi* Hartmann, *H. mirabile* Grassi (harboured by *Coptotermes sjöstedti* of French Guinea), and *H. hemigymnum* Grassi (harboured by *Coptotermes lacteus* of Australia). The chief differences between them seem to be in the arrangement of the spiral bands of flagella. According to Grassi (1917), *H. hertwigi* has very numerous bands, *H. mirabile* has only 12, while *H. hemigymnum* often has no bands over a large part of the posterior region of the body. Grassi's estimate of the number of bands in *H. hertwigi* is based upon Hartmann's Figure 44, which shows 20; but Hartmann's description and figures are not sufficiently accurate to warrant any definite conclusion regarding their number. Another of his figures (Fig. 46) is said by Hartmann himself to show 14 bands, but the drawing is not at all clear. The species of *Holomastigotoides* found in *Coptotermes formosanus* is similar in size to that described from the *Coptotermes* of Brazil by Hartmann; but owing to the incomplete and inadequate description which he has published, it is impossible for me to determine with certainty whether his species and mine are the same or different. From Grassi's brief descriptions it seems certain that his two species differ from mine, and I propose therefore to name my species *H. hartmanni*, provisionally regarding it as new.

Holomastigotoides hartmanni sp. nov. (Pl. XIII, figs. 43–52).

This is a large and plump form, commonly oval or elliptical in outline, and slightly narrowed at the anterior end. It usually measures 50–140 μ in length, but sometimes as much as 170 μ; its breadth being usually 30–80 μ, exceptionally attaining 100 μ. On the greater portion of the body a distinct and well-developed periplast is recognizable. The shape of the body is not changeable. (See Pl. XIII, fig. 43.)

Close spiral bands of deeply stainable minute granules, as seen in *Pseudotrichonympha* and *Microspironympha*, are found over the greater portion of the body, excepting a small area at the posterior end, equal to about one-ninth of the whole body. The bands contain the basal granules of the flagella, as in the other genera. The number of bands appearing on one side of the body varies from 30 to 50, according to the size of the organism. In the Brazilian species they seem less numerous and distributed more diffusely than in our forms, and in *Holomastigotoides hemigymnum* described by Grassi (1917) a large portion of the body remains free from bands, as mentioned above. The course of all of the bands may be traced to the anterior tip of the body, as in *Microspironympha*; but the number of them originating at this point seems to be inconstant in the present species, and varies from

8 to 16. The direction of the spiral is dexiotropic, as in *Microspironympha*, and thus the opposite of that in *Pseudotrichonympha*[1].

The surface of the body—covered by a well-developed periplast, as mentioned above—appears to be smooth, but good preparations reveal the existence of fine ridges running spirally in parallel with the bands of granules. The ridges and flagella in this organism are in close connexion, just as in the body of *Teratonympha*. The ridges are sharply pointed, though but little elevated, and are inclined strongly backwards, so that a shallow and narrow groove is formed behind each of them. The bands of granules are localized at the bottom of the grooves, and rather deep in the endoplasm, so that a small portion of the root of each flagellum is embedded in the protoplasm (Pl. XIII, fig. 53 *b*). The flagella are numerous and fairly long in this species, measuring 20–30 μ. Not only are the roots of the flagella embedded in the endoplasm, but their basal portions are also fixed on the surface of the body, just as in *Teratonympha*: they run backwards, lying fixed on the surface, until they reach the vicinity of the summit of the next ridge, where they become free. The ridges are commonly found depressed on the surface, so that the grooves behind them almost disappear and the characteristic structure is liable to escape the observer's eye.

The posterior end of the animalcule, somewhat metabolic as compared with the anterior, is destitute of spiral bands, as mentioned already, but is not naked. Here there are a large number of fine filaments, distributed thickly on the surface. They appear like cilia, but are distinguishable from the latter in several points. They seem brittle, less pliant than ordinary cilia, and slightly wavy. They are thickest at the middle portion and taper slightly towards both ends, and no structures such as basal granules are noticeable at their points of attachment. When observed in motion in the fresh state, they do not undulate together like ordinary cilia, but are swung independently in a peculiar manner, like fairly flexible filaments fixed at one end. It is possible that they are peculiar appendages like those described in *Parajoenia* by Janicki (1915), or they may be adherent micro-organisms, such as are often seen on other trichonymphids. They are slightly thicker and shorter than the flagella on the body wall. In the Brazilian species the posterior end, destitute of bands, seems narrower than in our forms; but in *H. hemigymnum* of Grassi, on the contrary, it is very wide. In these two species the posterior portion is apparently free from any appendages. Grassi described an area covered by "motionless cilia" (stereocilia) at the hind end of *H. mirabile*—his figure showing similar arrangements to those just described in *H. hartmanni*.

The greater part of the body of *H. hartmanni* is invested by a fairly tough periplast as described above. The endoplasm is specially compact under the body wall and is distinguished as a clear zone from the main portion, which is of looser constitution, and usually contains a large quantity of food débris.

[1] The spirals are drawn as right-handed by Grassi, and partly as right-handed and partly as left-handed by Hartmann—the latter being doubtless incorrect.

The nucleus is situated near the anterior end, embedded in a mass of special protoplasm, transparent, dense, and homogeneous, and staining somewhat deeply with eosin. In the young forms this peculiar protoplasm is only distinctly recognizable around the nucleus; but in large ones it is remarkably dense, and is also found strongly developed both in front of and behind the nucleus. In front it assumes the shape of a cone, reaching anteriorly to the tip of the body. At the sides and behind the nucleus, one, two, or three columns of it, gradually decreasing in thickness towards the hind end, are found hanging in the endoplasm (Pl. XIII, fig. 46). These columnar structures are remarkably distinct in some individuals and appear fibrous. Hartmann's description of the Brazilian species of *Holomastigotoides* contains no account of any corresponding structures, but quite similar structures are described and figured in *H. mirabile* and *H. hemigymnum* by Grassi. I am inclined to doubt the accuracy of Hartmann's observations on this point.

The nucleus of mature individuals appears usually elliptical in outline, measuring 20–26 μ by 10–15 μ, the major axis being situated transversely. The nuclear membrane is very distinct, and the special protoplasm immediately surrounding it is dense. The internal structure of the resting nucleus is somewhat peculiar in this organism. In some individuals a chromatic body, resembling a band or a spindle and consisting of deeply stained granules embedded in somewhat deeply stained ground substance, is found crossing the nucleus transversely (Pl. XIII, figs. 43 and 45). The granules are, in some individuals, large in size and few in number, while they are minute and numerous in others. In some cases the chromatic body is divided into two halves situated side by side (Fig. 46). It is also common that the chromatic substance appears as a small number of globular masses or rod-like bodies, stained rather intensely and homogeneously with iron-haematoxylin (Fig. 44). The nuclear sap is not structureless and homogeneous as in the preceding forms, but fine granules, faintly stainable with iron-haematoxylin, are present in it. These granules are found, as a rule, apart from the chromatic bodies and distributed chiefly at the periphery of the nucleus. The situation of the chromatic bodies in the nucleus is noteworthy in this species. When they form a band or spindle, it lies transversely, as mentioned above, and is found commonly connected with the nuclear membrane at both its ends: when two masses are present, they are situated symmetrically at both sides, and they are also commonly kept in connexion with the membrane at symmetrical points. At the point of connexion of the chromatic bodies with the membrane a minute granule is frequently recognizable.

The process of division of the nucleus in this species appears somewhat peculiar. The nucleus, when going to divide, changes its position. It is drawn near one side of the body, and usually becomes narrowed towards the point drawn near the body wall. These changes are sometimes not so distinctly seen, or are unnoticeable because of the position of the nucleus under the microscope, but they are very conspicuous in some cases. In the nucleus

entering into division, the chromatic granules are small and numerous, embedded in the ground substance, and arranged in the form of a thick spindle (Pl. XIII, fig. 47). A short strand makes its appearance at the pole lying under the body wall, and the above-mentioned mass of chromatic granules is found hanging in the nuclear sap, attached to the nuclear membrane just at the place where the short strand is formed. The chromatin granules break up and become rearranged to form a certain number of looped threads with their ends attached to the ends of the strand. The strand and the threads now become gradually more and more distinct; the strand becomes thickened and elongated, though rather slightly, and the threads become thick and uniform chromosomes (Pl. XIII, figs. 48 and 49). In early stages there are usually two long threads and some shorter ones; the former assume the form of U- or V-shaped loops, fixed at both ends of the strand, while the latter are found hanging from either end. The former threads are those that persist as chromosomes through the later stages. I have counted one to three additional threads in nuclei at a rather advanced stage in division, but they disappear later. The chromosomes having the shape of a loop divide transversely into two at the middle, and each limb becomes a daughter chromosome. Pl. XIII, fig. 50 represents one of the specimens in the most advanced stage which I could study. In this stage the strand measures 4–5 μ, and is fused with the nuclear membrane. It is fairly thick, pointed at both ends, and stained deeply with iron-haematoxylin, showing occasionally a fibrous structure. The individual shown in Pl. XIII, fig. 51 is also in the most advanced stage, and the daughter chromosomes are distinct, but the strand is invisible, owing to its disposition. The next stage which I have been able to find shows the already completely separated daughter individuals. I could not follow accurately the later stages of division. It will not be unreasonable, however, to suppose that the strand becomes further elongated, as in the preceding genera; but I am inclined to suppose that the nucleus is quickly divided after the above stage is reached.

In young animalcules lately divided, the nucleus is round and the two daughter chromosomes are found hanging side by side from the anterior pole. In many cases, the greater portion of the chromatin becomes, sooner or later, aggregated into an oval mass, hanging in the nuclear sap at two points, and then disintegrates, forming the characteristic structure of the resting nucleus, with a rather feebly stained ground substance containing intensely stained granules embedded in it (Pl. XIII, fig. 52). As the animalcule grows, the nucleus becomes gradually flattened and elliptical in outline, and the points of attachment of the chromatic body become separated gradually from each other. Parallel with the alteration of the position of the points of attachment to the membrane, the shape of the chromatic body becomes also altered so as to form a band or spindle, which may be divided into two halves. In some cases, the daughter chromosomes remain in a rod-shaped condition for a long time; or they may become divided into two, three, or four pieces, and persist in that condition.

Hartmann's statements regarding the division processes of the Brazilian species appear very doubtful to me. Basing his opinion upon a small amount of material, he concluded that both amitosis and mitosis may occur. As for the latter, he figures several stages interpreted as prophases, in which chromatin granules are distributed in the nuclear sap in the form of irregular threads. They differ strikingly from the prophases of mitosis in our forms. As for the amitosis, he described two stages, one interpreted by him as an early stage, and the other as a young form just after division. Specimens with the nucleus of the same type as that interpreted by him as an early stage of mitosis are frequently met with in our species; but these are merely forms with resting nuclei of a special type, as mentioned above, and have nothing to do with division. The forms interpreted by him as the young have a structure so remarkably different from the parental form, that no one can recognize their genetic connexion, in the absence of intermediate forms. Hartmann, however, neither discovered any forms showing an intermediate structure nor gave any evidence to indicate their genetic connexion. I have failed to find any forms with a similar organization in the Japanese termites.

VI. *Spirotrichonympha.*

The genus *Spirotrichonympha* was introduced by Grassi (1911) for the flagellate originally wrongly referred by him (1892, 1893) to Leidy's genus *Pyrsonympha*. The type species of this genus is *Sp. flagellata*, described by Grassi in his work with Sandias (1893). It has since been renamed *Leidya metchnikovi* by França (1916). (See França, 1918, and Grassi, 1917.) In his recent work, Grassi (1917) distinguishes three species of this genus; one of them with two varieties. These are *Sp. mirabilis* (in *Porotermes adamsoni* from Australia), *Sp. flagellata* var. *schedorhinotermitis intermedii* (in *Schedorhinotermes intermedius* from Australia), *Sp. flagellata* var. *coptotermitis lactei* (in *Coptotermes lacteus* from Australia), and *Sp. elongata* (in *Schedorhinotermes intermedius*). In my former paper (1917), I described an organism harboured by *Coptotermes formosanus* under the name of *Cononympha leidyi*, establishing a new genus for it, because I could not identify it as a species of *Spirotrichonympha*, the characters of which genus were given very briefly in Grassi's work of 1911. But his recent work made me clearly understand that my organism belongs to this genus. The Japanese species appears to differ, however, from all species reported.

Spirotrichonympha leidyi sp. nov. (Plate XIII, figs. 41 and 42).

This is the smallest of the forms found in *Coptotermes formosanus*, and measures 15–50 μ in length and 8–30 μ in breadth; that is to say, the largest individuals hardly exceed in size the smallest forms of most other species found associated with them. This organism is also clearly distinguishable by its shape from the other forms in the same host. It has usually the shape of a

tall cone, the posterior or basal part of which is slightly convex. The body wall is more or less rigid at the sides, chiefly owing to the character of the bands of flagella described below, only the posterior basal part being somewhat metabolic.

Spiral bands of deeply stained granules, containing the basal granules of the flagella—such as are seen in *Microspironympha* and *Holomastigotoides*—are found over the greater portion of the surface. The number of the bands appearing on one side varies from 10 to 20, and their course is rather oblique: but this does not represent the true number present, which I have not yet succeeded in determining. The disposition of the bands is peculiar in this genus: they are not found directly under the periplast, but are situated somewhat more deeply in the protoplasm, a portion of the root of each flagellum being embedded in it. The above character is especially marked at the anterior end, and becomes less distinct towards the posterior; that is to say, the basal granules of the flagella lie deeper at the anterior end of the body. The flagella are fairly long, measuring 10–16 μ, and run rather sharply backwards. The direction of the spiral of the bands is the same as in the preceding genus, namely dexiotropic or right-handed; that of the forms studied by Grassi is drawn also as right-handed by him. The posterior portion of the body is free from any appendages.

The nucleus is oval, and is found in the middle region of the body. Its internal structure is of a type resembling that of *Pseudotrichonympha*, consisting of clear and structureless ground substance and chromatic bodies arranged in an irregular network or scattered under the nuclear wall and in the ground substance. One of the most characteristic features of this form is a conical structure at the anterior region of the body, consisting of dense and homogeneous protoplasm, and apparently of the same substance as the particular protoplasm surrounding the nucleus in *Holomastigotoides* and *Microspironympha*. It appears most dense in its consistency and most distinct in its outline at the anterior part, becoming gradually less dense and less distinct towards the posterior. This mass of protoplasm is almost invisible near the nucleus, but I think it probable that it there merely becomes thinner, and the nucleus is embedded in it.

As has been pointed out by Grassi (1911 and 1917), the grounds for Hartmann's view that a form closely resembling this was the young of "*Trichonympha hertwigi*" were certainly insufficient. As regards the genetic connexion of his "young form" and "male form," he himself said that all intermediate types were not made out—especially as regards the arrangement of the flagella, no intermediate forms having been found. As regards the structure of the anterior end, he remarked that some intermediate types were observed, but neither descriptions nor illustrations of these forms were given in his paper. In our organisms, no forms showing characters intermediate between this form and *Pseudotrichonympha* were met with: and as the rows of flagella of *Spirotrichonympha* are in dexiotropic spirals, while those of *Pseudotrichonympha*

are laeotropic, it is hard to imagine that the one can grow into the other. As for the relationship between his "young form" and "female form" (*Holomastigotoides*), there are some points of resemblance in their organization. But they differ distinctly in the disposition of the basal granules of the flagella and the structure of the nucleus. No intermediate forms were recognizable, and the existence of a genetic connexion between the two is hardly conceivable.

Although *Spirotrichonympha leidyi* occurs in all the individuals of *Coptotermes formosanus* which I have examined, it is not so abundant as some of the other species. Unfortunately I have been unable to find any dividing forms, or other stages in development.

VII. *Holomastigotes.*

This is a genus established by Grassi (1892), and described in his work with Sandias (1893), for a small organism found in Italian termites. He defined the genus more accurately later (Grassi, 1911) and published further details and figures in 1917. Forms closely resembling or probably identical with it are also found among the figures of the American trichonymphids, given by both Leidy (1881) and Porter (1897), but erroneously regarded by them as possible young stages of *Trichonympha*. An organism described and figured by Comes (1912, Fig. 1) as a "young *Pyrsonympha* with resting nucleus" also appears to belong to this genus.

Closely similar forms also occur in the species of *Leucotermes* of Japan proper and of Formosa. The shape is variable in our organisms, and no sharp morphological distinction can be drawn between them and the Italian or the American forms. As regards the finer details of structure, it is not possible to determine whether they show any differences or not, owing to the insufficiency of previous descriptions. I propose, therefore, to call our form provisionally by the name of the Italian species described and figured by Grassi (1917) in his recent work.

Holomastigotes elongatum Grassi (?) (Plate XIII, figs. 39 and 40.)

This is a small and commonly fusiform or lanceolate form, measuring 15–45 μ in length and 10–15 μ in breadth. According to Grassi (1893) the type species attains a length of 70 μ and a width of 24 μ. The forms which I have studied thus appear to be considerably smaller, and should therefore, perhaps, be regarded as an independent variety. Grassi (1917), however, in his recent work does not state the dimensions of his species; but from his figures, of which the magnification is recorded, it appears that the organism may be considerably smaller than his original account would lead one to suppose. The body usually tapers towards the posterior end, and the anterior end usually projects slightly, forming a mammiform eminence. In this feature it appears to differ from the forms described by Grassi. The animalcule is, however,

variable in form, and, when alive, is frequently club-shaped or pear-shaped.

The periplast is fairly well-developed and distinct ridges are found on the body wall, winding spirally from the anterior tip to the posterior end. The direction of the spiral is also right-handed in this form[1]. The edge of each ridge seems rather sharp and stiff, especially in the anterior region, so that it appears as though supported by a cord. The number of ridges seems to be eight. They are sloped backwards and at the posterior edge of each of them a shallow but distinct groove is formed behind; also a distinct row of basal granules of the flagella is there found, embedded in clear protoplasm. These spiral ridges are thus identical with the spiral ridges in *Holomastigotoides*, and represent the same type of structure as is seen in the well-developed transverse ridges in the body wall of *Teratonympha*. The disposition of the flagella is also identical with that of *Teratonympha* and *Holomastigotoides*. The basal end of each flagellum is adherent to the surface of the ridge and becomes free at its edge, so that the surface of the body between the ridges or folds appears beautifully and densely striped (Pl. XIII, figs. 39, 53 *c*). The free portion of the flagellum measures 10–15 μ. The endoplasm is rather clear, and usually contains a large number of small refringent globules ("round corpuscles" of Grassi), taking haematoxylin and eosin somewhat deeply (Pl. XIII, fig. 40). It is peculiar to this form that wood particles and other food débris are not usually present in the endoplasm.

The nucleus is situated at a short distance from the anterior tip, and is usually pear-shaped. There is a mass of peculiar dense protoplasm, as seen in the preceding genera, at the anterior tip of the body, and the nucleus is usually found embedded in it. The internal structure of the nucleus is somewhat peculiar in this form. It consists of a structureless ground substance, staining rather deeply with iron-haematoxylin, and having several chromatin masses embedded in it.

I have found no other stages of development than that just described, and I have encountered no dividing forms in my preparations.

(2) Pyrsonympha Series.

This group embodies a number of forms clearly distinguished from the members of the previous series. In them the body is not invested with numerous flagella resembling cilia, but is provided with peculiar slender flagellum-like cords, four or eight in number, which I propose to call "flagellar cords" ("undulating lines" of Leidy, and "contractile cords" of Porter). It is true that, in some forms, delicate appendages are found distributed over the body, giving it the appearance of being invested with cilia; but these appendages are quite different in nature from ordinary cilia or flagella, and are something like those seen at the posterior end of *Holomastigotoides* and

[1] Drawn right-handed by both Grassi (1917) and Porter (1897, Plate III, figs. 30–32, 35, 36).

in some other trichonymphids. Some of them are doubtless adherent micro-organisms, but some are probably peculiar appendages similar to those described in other Protozoa by such names as "bristles," etc. One other characteristic structure of the members of this group is an elastic filament, which I shall call the axial filament ("undulating cord" of Leidy and "flagellum" of Porter), lying in the protoplasm and stretching from the anterior tip of the body to the posterior end or its vicinity. The flagellar cords arise at the anterior tip of the axial filament and run spirally backwards, attached to the body wall, and become free at the posterior end. The direction of the spiral is always left-handed (laeotropic). Porter described the direction of the spiral of the cords in the American species as right-handed, but the figures given by him are drawn as left-handed. I think it probable that his description is a mistake, and his figures are correct. The drawings of Grassi are left-handed[1].

This group comprises two genera of the American authors, *i.e. Pyrsonympha* and *Dinenympha*, two of the three oldest genera established for the Protozoa of American termites by Leidy in 1877. One species was described by him under each of these genera, and figures were given in his later work (1881). The same names were applied by Porter (1897) to similar forms, studied by himself in the same country. Grassi (1893), in his work with Sandias dealing with the Italian termites, described two species which he referred to the above two genera. The form referred by him to the genus *Pyrsonympha* is one thickly covered with flagella, differing distinctly from the type of the genus described by the American authors. It is thus unquestionable that he made an error in the application of the generic name. He has himself corrected this mistake in later publications (1911, 1917); and the organism which he originally called by Leidy's name is now the type species of the genus *Spirotrichonympha*, established by him (Grassi, 1911). The forms described by Grassi (1893) under the name of *Dinenympha gracilis* differ also from the forms to which Leidy gave that name, and represent several types with the characteristics of both *Pyrsonympha* and *Dinenympha* of the American authors. Originally Grassi did not draw any distinction between Leidy's two genera; but it appears that he is now of the opinion that the type forms of the two genera are merely different stages of growth of a single form; and in a review of the genera given in his work which appeared in 1911, the two were united into one genus, *Dinenympha*. Comes (1910 *a*) seems to be of the same opinion as Grassi, for he put forward the view that various forms referable to Leidy's two genera represent different stages of development of *Dinenympha gracilis*. The organism which he described as "*Pyrsonympha*" subsequently (1912) is in reality *Spirotrichonympha* and *Holomastigotes*. However, he has also described an organism closely similar to Leidy's *Pyrsonympha*, referring it to the new genus *Lophophora* (1910). Grassi (1911) regards this as a stage in development of the same organism, and refers it

[1] Grassi and Sandias (1893), "*Dinenympha gracilis*," Plate V, figs. 11–14.

to the genus *Dinenympha*. Recently Zulueta (1915) has published a paper on "*Dinenympha gracilis*," but the organism which he describes is clearly the same as that which Leidy called *Pyrsonympha*.

In *Leucotermes* of both Japan proper and Formosa, forms belonging to this group are found in large numbers. They are so numerous that they far exceed the members of the preceding group in number. They present such remarkably diverse types of organization that it is clear they cannot be referred to one or two species, as has been done by previous authors in the case of the American and Italian forms. Some of our forms are of such peculiar organization that they are easily distinguishable from each other, but there are several which are not so readily distinguishable, and their classification demanded much time and work. I finally determined that they are to be referred to eight species—one of them doubtful.

Previous authors do not seem to have been sufficiently accurate in classifying their species. The illustrations given by the American authors are more or less detailed; and they show clearly that several different forms, which undoubtedly present characteristics proper to distinct species, are included under a single specific name. It can be clearly recognized, moreover, that the forms referred by Porter (1897) to *Dinenympha* are of a different character from the original forms described under this name by Leidy. As for the works of Grassi (1911), Comes (1910, 1910 *a*, 1912), and Zulueta (1915), both their descriptions and illustrations are so incomplete that the identity of their forms with the others is hardly ascertainable.

The classification of the organisms of this group described hitherto is thus in a very confused state; and this has puzzled me very much in the adoption of specific names. I have finally been compelled to call all the species occurring in the Japanese termites by new names.

Our forms may be divided into two groups, which I think to represent the characteristics of *Pyrsonympha* and *Dinenympha* of Leidy. The differences found between these two groups, however, were not thought to be so distinct as to justify their reference to different genera: so I have marked the distinction by classifying them as two subgenera of a single genus. Thus I agree with Grassi (1911) in the conception that Leidy's two genera should be united into one, but I cannot concur with him in the adoption of the generic name. I propose to adopt *Pyrsonympha*, not *Dinenympha*, as the generic name, for the following reasons: the characters of the group are more typically and distinctly represented in *Pyrsonympha* than in *Dinenympha*, and the former name stands prior to the latter in the original description of Leidy (1877). I agree with Grassi (1911) that *Lophophora* Comes is a synonym: and I may point out that this name cannot be used in any case, as it is already preoccupied—having been proposed on at least two previous occasions for insects.

The chief points of distinction of the two subgenera are as follows:

I. **Pyrsonympha.** The body very large, large, or medium sized; piriform, club-shaped, or screw-like. The body is twisted and the flagellar cords wind round it; some forms being twisted into a screw, but not curled, as a whole, into a spiral. The axial filament hangs in the endoplasm, ending freely in it at the posterior end. Two species are distinguished:

(1) *P. grandis* sp. nov. (in *Leucotermes speratus* and *L. flaviceps*).
(2) *P. modesta* sp. nov. (in *Leucotermes speratus* and *L. flaviceps*).

II. **Dinenympha.** The body small and slender; club-shaped or lanceolate. Whole body curled into a spiral. The axial filament distinct or indistinct: in the former case its posterior end is held fixed at the posterior tip of the body. Five species and one doubtful species are distinguished:

(1) *P.* (*D.*) *exilis* sp. nov. (in *Leucotermes speratus* and *L. flaviceps*).
(2) *P.* (*D.*) *rugosa* sp. nov. (in *Leucotermes speratus*).
(3) *P.* (*D.*) *nobilis* sp. nov. (in *Leucotermes speratus*).
(4) *P.* (*D.*) *leidyi* sp. nov. (in *Leucotermes speratus* and *L. flaviceps*).
(5) *P.* (*D.*) *parva* sp. nov. (in *Leucotermes speratus* and *L. flaviceps*).
(6) *P.* (*D.*) *porteri* sp. nov. (?) (in *Leucotermes speratus* and *L. flaviceps*).

I. *Pyrsonympha.*

The organisms belonging to this subgenus vary remarkably in size, and their shapes are, moreover, somewhat conspicuously changeable. Thus I have been greatly puzzled in determining the exact number of species to which our forms are to be referred. As a result of protracted observations, I have arrived finally at the conclusion that there are really two sets of forms, each set referable to an independent species. Both of these species are common in *Leucotermes speratus* of Japan proper and *Leucotermes flaviceps* of Formosa.

(1) *Pyrsonympha grandis* sp. nov. (Plate XIV, figs. 54–64).

The forms of this species, like the others of the same subgenus, differ from the members of the preceding series in their mode of life in the intestine. They do not always live freely in the lumen of the gut, but a large number of individuals are found attached to the wall and hanging in the cavity. The forms attached to the wall are usually so numerous that they far exceed the unattached forms in numbers. They are found closely assembled and hang perpendicularly from the intestinal wall, assuming the shape of a club, rather long and straight or slightly curved. In individuals living freely in the lumen, the body is shortened and thickened, especially at the posterior region, so as to assume the shape of a flask, with a rather large body and a somewhat slender neck. Frequently the organism is so thickened that the body becomes piriform; but sometimes it is rounded anteriorly, so that the body appears oval. The same mode of life was noticed by Porter in the American species. According to him, the young forms are found attached to the intestinal wall

and the mature ones invariably swim freely in its cavity. It is also the case in our forms that most individuals attached to the wall appear to be small, but attached individuals of large size are also found quite commonly. It is by no means rare, moreover, to find small forms swimming freely in the cavity of the gut. In the club-shaped forms hanging from the wall, the body is almost straight or slightly curved, but the course of the flagellar cords on the body wall is distinctly spiral, indicating clearly that the body is twisted in various degrees. In the forms living freely in the intestinal cavity, the body is not only twisted but also spirally wound as a whole. Forms hanging from the wall do not remain quiet, but continue to move gently. Free-swimming forms usually display vigorous motions; and though they do not change their position markedly, the anterior portion is commonly swung very actively in all directions.

The dimensions also vary considerably in this species. Individuals measure usually 40–150 μ, occasionally as much as 170 μ, in length, and 19–40 μ, sometimes up to 50 μ, in breadth. According to Leidy (1881), *Pyrsonympha vertens* measures 100–160 μ in length, but Porter describes specimens attaining a length of 275 μ and more. Comes (1910 *a*, 1912) and Grassi (1893, 1911) give no measurements of their forms, whilst Zulueta (1915) states that his species (called "*Dinenympha gracilis* Leidy," but really a *Pyrsonympha*) measures from 18 μ to 90 μ or more.

The axial filament is very well-developed in this species. It hangs from the anterior end of the body in the endoplasm and lies quite free from the body wall throughout its entire length. It is thick, and oval or elliptical in cross section at the anterior portion. Towards the posterior end, it becomes gradually thinner and slender. Its posterior extremity is not connected with any part of the body wall, but ends freely in the endoplasm. In young individuals, the filament is slender and stains homogeneously with iron-haematoxylin and also with eosin. In large forms, however, it is thick at the anterior portion, and the posterior is usually found split into two, three, or sometimes more, slender filaments (Pl. XIV, figs. 54 and 55). The anterior portion of the thick filament is commonly not homogeneous, and a deeply stainable wall and somewhat feebly stainable contents are distinguishable, though the boundary between these parts is indistinct. In some individuals a small portion at the anterior extremity shows an appearance as if consisting of several slender cords, taking dyes intensely, arranged regularly on the surface of a rod staining very faintly (Pl. XIV, fig. 57).

As regards the axial filament, some differences are recognizable between our forms and the American ones. The filament does not end freely at its posterior extremity in the American forms, but is fixed at the hind end of the body; and it seems, moreover, to remain simple, never splitting even in the later stages of growth.

In *Pyrsonympha grandis* the anterior extremity of the axial filament is more or less abruptly pointed, and it is at this pointed extremity that the

characteristic flagellar cords arise. No special structure such as a basal granule is recognizable at the point of connexion of the cords and the filament. In the majority of cases the pointed extremity of the filament is found at the anterior tip of the body, and the cords arise directly from it at that point; but not unfrequently the pointed end of the filament lies a little within the endoplasm, and is then provided with a rather thick stalk connecting it to the anterior tip of the body, the cords arising from the end of the stalk (Pl. XIV, fig. 57). The number of the cords is four in the young forms and eight in the mature ones. They run backwards, attached to the surface of the body, to the posterior end, where they become free. Porter remarked that the cords of the American species "can be traced to the posterior extremity of the animal, and thence back again on the opposite side of the animal to the anterior end." This is not true of our forms. The free ends of the cords, on the contrary, are fairly long and distinct, and never escape the eye of a careful observer. The cords run in a left-handed spiral round the body. They are rather straight in small forms, while they are remarkably wavy in large forms, and the larger the body, the more conspicuous is their winding. The surface of the body is not even, but each cord is situated on a ridge. In small forms the ridges are rather indistinct; but they are distinct in large forms, especially at the anterior end, where they frequently appear as rather flat and elevated membranes resembling the undulating membrane of *Trypanosoma*. In the living animal the cords and their membranes display a continuous and beautiful rippling motion, the ripples passing from before backwards.

In the majority of forms, the body is naked and destitute of any kind of appendages, but rarely large individuals invested rather thickly with filamentous bodies are met with. In the American species studied by Leidy some are naked, while others are thickly "ciliated," and those studied by Porter seem to be invested thickly without exception. It seems certain that the filamentous appendages seen in our forms are not real organs of the animals themselves.

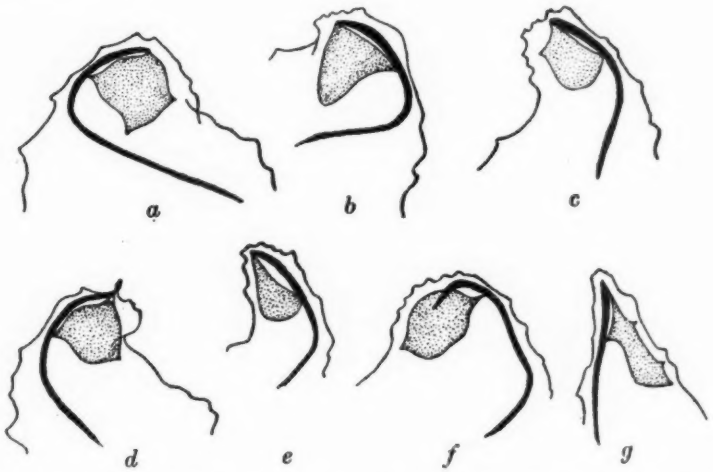
Individuals lately detached from the gut wall are frequently provided with a knob-like structure, stained quite as deeply as the axial filament, at the anterior end of the body, where the pointed end of the filament is fixed. The knob is usually situated at the tip of the axial filament, but sometimes a short peduncle connecting them is observable. In some forms the knob assumes the shape of a sphere or an ellipsoid, and is unbroken in its outline (Pl. XIV, fig. 54); but in others it is not smooth but prickly at its distal end (Pl. XIV, figs. 57 and 62). Long and distinct splinter-like filamentous structures are also frequently visible at this end (Fig. 56). This split or frayed-out structure is variable in size and shape; it is commonly single, but sometimes two or more are found. As regards the function of the knob, I am of the opinion that it serves, when the animalcule is attached to the layer of chitinous substance investing the inner surface of the intestine, to preserve a firm connexion.

It seems highly probable that the knob itself is made of the substance of the axial filament. According to Porter's description, there is, in the American forms, a knob and a long filament ("peduncle" in his terminology), and the latter is inserted deeply into the intestinal wall. Sections of the intestinal wall of our termites were carefully studied, but I failed to recognize the existence of any structure corresponding to the "peduncle" of Porter. The splinter-like filaments in our forms are no other than artifacts, being produced artificially in preparing the animalcules for examination; and they are undoubtedly merely fibrous fragments or strips of the chitinous lining of the intestine. Similar structures to those just described have been observed in a species of *Pyrsonympha* by Comes (1910), but his interpretation of them is very different. He regards the frayed-out chitinous end as a tuft of consolidated flagella, and the knob as a blepharoplast: and proposes on account of this peculiar condition of the "flagella" to place the organism in a new genus *Lophophora*. As already noted, I agree with Grassi (1911) that this name should be regarded as a synonym of *Pyrsonympha* (*Dinenympha* of Grassi).

The nucleus is rather large and is situated at the anterior end of the body. It does not lie freely in the endoplasm but is closely connected with both the axial filament and the body wall, so that its shape is subject to modification, according to the state of contraction or extension of the anterior portion of the body. In the small club-shaped forms with slender bodies, the nucleus is also club-shaped and lies closely along the axial filament (Pl. XIV, fig. 54). In the large sized forms the nucleus is piriform, or rather triangular in outline, invariably much narrowed, or pointed, at the anterior pole. The nucleus becomes triangular, quadrangular, or semicircular in outline when the anterior part of the body is thickened. The anterior pole of the nucleus is found at the anterior tip of the body or in its vicinity, and it can be clearly seen in many organisms that the nucleus and the axial filament are kept in close connexion at their anterior ends. In many cases, the contour of the nucleus is smoothly rounded, except at the anterior pole; but in individuals abnormally thickened anteriorly it appears peaked at a few other points, and then it can be clearly seen that it is not only joined to the axial filament at another point, but also at one, two, or sometimes more points to the body wall. In Text-fig. C, *a–g*, several forms showing the modes of attachment of the nucleus are depicted. In the majority of cases, the peaked points of the nucleus touching the axial filament are directly attached to it. But in exceptional cases, the pointed anterior pole of the nucleus is found somewhat separated from the tip of the filament and connected with it by means of a thread. Once I met with a specimen in which the nucleus was rounded at its anterior tip, and connected with the tip of the filament by a funnel-like membrane (Text-fig. C, *d*). It thus seems probable that it is not the nuclear membrane itself that comes in contact with the filament, at least at the anterior pole, but that there is a membranous structure or sac, which invests the nucleus very tightly, and is kept in connexion with the axial filament and the body

wall at several points. As remarked above, the number of peaks visible on the nucleus is variable, depending on the condition of the body, and it is doubtful whether the points of attachment of the nucleus are constant in position or not.

The position and the shape of the nucleus seem to be quite different in the American and Southern European species of *Pyrsonympha*. In these the nucleus assumes an oval or an elliptical shape, and is situated away from the anterior tip of the body and not so closely connected with the axial filament. No description of the attachments of the nucleus to other parts of the body are to be found in the works of previous authors (Leidy, Porter, Grassi, Comes, and Zulueta). Some of the figures given by them, however, seem to indicate the existence of a structure similar to, but less developed than, that of our forms.



Text-fig. C. Anterior portion of *P. grandis*, showing various conditions of the axial filament and the nucleus. Drawn with camera lucida. ($4 \times \frac{1}{12}$.)

The internal structure of the nucleus of *Pyrsonympha* is quite different from that of the trichonymphids. It is of a type resembling that of the gregarines and coccidia, consisting of a fine and distinct achromatic network with minute chromatin granules, and one, two, or more large chromatic bodies, or karyosomes, situated centrally and usually surrounded by a clear space. The chromatic body has a vacuolate structure, and closely resembles the karyosome of gregarines and coccidia, but is often somewhat rough in its contour (Pl. XIV, figs. 54–56).

The endoplasm appears more or less homogeneous and somewhat compact in consistency, and rather closely resembles that of *Microspironympha* and *Holomastigotes*. In the small forms the endoplasm appears rather clear; but

in the large individuals it is frequently vacuolated, and great numbers of globular bodies of a peculiar kind are commonly found in it. These globules are rather refractive in fresh specimens, and stain with eosin somewhat deeply, but faintly with iron-haematoxylin[1]. As in many members of the preceding group, food débris is usually present in the protoplasm, but consists not only of vegetable matters (wood, etc.) but also of the bodies of other animalcules, such as small individuals of *Dinenympha*. Animalcules ingested into the endoplasm are found rounded and shrunk into spherical masses, and various stages of their disintegration, probably due to digestion, can be recognized.

Dividing forms were met with very rarely in this species, and I was barely able to make out the chief stages of the process, after an eager search through large numbers of termites in both Japan proper and Formosa. Division seems to take place invariably in the forms living freely in the lumen of the gut. In individuals going to divide, the body becomes rounded and the axial filament detached from both the nucleus and the body wall and set free in the endoplasm. The nucleus becomes oval or round in shape, and probably separated from the body wall, except at its anterior pole, so that it remains attached at only this one point, from which the flagellar cords arise. The nuclear network now disappears, the karyosome breaks up, and globular masses of chromatin make their appearance scattered in the almost structureless nuclear sap (Pl. XIV, fig. 58). Thick rod-shaped chromosomes are now formed from the chromatin, and a mitotic figure, somewhat different from that seen in the trichonymphids but resembling rather closely that of metazoan cells, makes its appearance. No structure corresponding to the strand seen in all forms of the preceding series is visible; but a distinct achromatic spindle is formed, with the chromosomes arranged at its equator (Pl. XIV, fig. 60). The chromosomes divide transversely, and the daughter groups move towards opposite poles in due course. The nuclear membrane persists throughout the entire process, and minute centrioles can be made out at the poles of the spindle in the later stages.

The flagellar cords remain connected with the nucleus, four of them being found attached to each pole of the spindle (Pl. XIV, fig. 61). Once I noticed a short fibrous strand on the membrane of the nucleus in an early stage of division (Pl. XIV, fig. 59). Unfortunately I have been unable to ascertain its origin and fate, or to determine its relation to the ends of the cords; but I am inclined to believe that it represents a stage in the formation of the achromatic spindle.

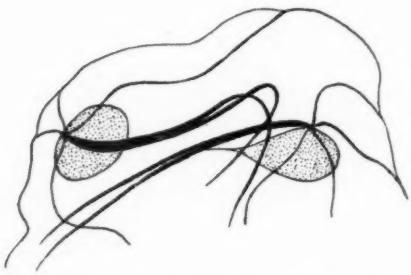
The axial filament gradually degenerates in the endoplasm and disappears before the division of the nucleus is completed. Shortly after the daughter nuclei are formed, two new axial filaments can be seen, one attached to each of them (Text-fig. D). As regards the origin of the new axial filaments I have

[1] The vacuoles are usually present in only the posterior two-thirds of the body. It was the presence of such vacuoles which apparently led Comes (1910) to bestow the specific name *vacuolata* upon the *Pyrsonympha* which he regarded as belonging to a new genus *Lophophora*.

nothing to say with certainty. At the time when they first become visible in my preparations, no centrioles can be seen at the points where the flagellar cords join the nuclear membranes.

I have found no other processes of multiplication than those just described. The stages of division of *Pyrsonympha* (incorrectly called *Dinenympha gracilis*) described and figured by Zulueta (1915) agree closely, so far as they go, with those which I have observed. On the other hand, the remarkable cycle of development described by Comes (1910 *a*, 1912) in similar forms is very different from anything that I have seen. This author describes "males" and "females," "conjugation," "multiple fission," and a peculiar development in the salivary glands of the termites. I agree with Grassi and Zulueta in believing that no such stages exist, and that Comes' interpretation of his findings is incorrect.

As mentioned already a remarkably large number of individuals are found attached to the intestinal wall: and these are not only young and small forms,



Text-fig. D. A late stage of division of *P. grandis*, showing the flagellar cords and newly formed axial filaments. Drawn with camera lucida. ($4 \times 1\frac{1}{2}$.)

but large and mature ones as well. In studying these forms I often noticed gigantic piriform or fusiform individuals with a conspicuously jagged surface, distinguishable at a glance from the normal forms. On making a special search for these peculiar forms in a large number of termites, I was able to find not a small number of them, representing several types of structure. At a time when I had insufficient material, I was inclined to imagine that they represent stages in a particular mode of multiplication. The fact that a large number of young forms are found hanging from the wall in close association seemed to favour this supposition. But on studying more material, I soon realized that they represent successive stages in a process of physiological degeneration.

In the early stages of degeneration the nucleus becomes detached from both the axial filament and the body wall; in some forms it is broken in two, and one half remains connected with the filament at its normal position, while the other is set free in the endoplasm (Pl. XIV, fig. 62). The endoplasm itself

appears denser, but contains no food débris, while a large number of minute globular bodies, staining deeply with eosin and faintly with iron-haematoxylin, are found scattered in it. The nucleus, suspended in the endoplasm, becomes gradually hypertrophied and disintegrated. The axial filament also disintegrates by a somewhat peculiar process; it does not become detached from the anterior end of the body—as it does during division—but remains fixed in its normal position and becomes strongly curled into a rather regular spiral (Pl. XIV, fig. 63). In degenerating nuclei the network becomes indistinct, until finally it vanishes entirely. The karyosomes break up into irregularly shaped and ill-defined masses of chromatin, isolated from each other or fused together. In Fig. 64 is shown a nucleus at an advanced stage of degeneration, with several clumps of chromatic substance, stained rather faintly with haematoxylin, embedded in a coarsely granular ground substance. The clumps stain more faintly and their contour gradually becomes less distinct. Hand in hand with the above changes, the nuclear wall becomes thinner and the globules in the endoplasm increase in number, especially in the vicinity of the nucleus. The filament also becomes gradually indistinct, and soon disappears, apparently dissolving in the endoplasm.

The process of physiological degeneration has been more or less thoroughly investigated in artificially cultivated individuals of several forms of free-living Protozoa by R. Hertwig, Prandtl, and others. As regards tissue-parasites, there are also some descriptions by Schaudinn and Léger of degeneration observed in Coccidia. Regarding the other intestinal Protozoa, however, few accounts have hitherto been given. Moreover the process in *Pyrsonympha* shows some interesting peculiarities. How is it that such an uncommon phenomenon is seen in this species? This is a question of considerable interest, and I am of the opinion that the answer is to be sought in certain peculiarities both of the protozoon itself and also of the intestinal wall of its host. In the other forms leading a free-swimming life in the cavity of the gut, individuals inclined to degenerate cannot long remain in the intestine, but sooner or later are cast out. In *Pyrsonympha*, however, the body is firmly united to the intestinal wall, and the mode of attachment is peculiar. It is not a cell of the intestine, but its covering layer of chitin, to which the animalcules are attached; and it is not by means of the soft part of the body, but by a special rigid structure, that the connexion is maintained. The attachment is thus purely mechanical, and does not depend upon the vital activity of the flagellate. It seems, therefore, highly probable that the animalcules may remain fixed in position, regardless of their state of vitality; so that if they die or degenerate, they do not pass out of the body, like the forms living freely, but remain, though dying and even dead, still suspended from the intestinal wall.

(2) *Pyrsonympha modesta* sp. nov. (Plate XIV, figs. 65 and 66).

The individuals of this species resemble the young forms of the preceding, but show some differences in their shape and some other structures. They are fairly small in size, measuring 30–80 μ in length, and are commonly pear-shaped. The nucleus is oval or round, and is situated at the anterior end. The flagellar cords run almost straight, and the ridges on which they are situated are commonly indistinct, except in much twisted forms. The cords themselves are confined, at the anterior end, to one side of the body, the other being free from them. The axial filament is more slender than in any individuals of the preceding species, and is always single. The endoplasm is usually clear, though it frequently contains globular bodies. A peculiarity of this form is that the body is often conspicuously twisted, with the anterior end bent back into a peculiar form, so that it resembles a screw (Pl. XIV, fig. 66). In such individuals the bare area—noted above—at the anterior end is seen to lie in the inner angle of the bend.

Dividing forms are also rare in this form, and the process of division appears, as far as I could conjecture from my material, to be of a type identical with that of the preceding species.

II. *Dinenympha.*

This subgenus embodies a number of species, distinguished from the preceding forms by the shape of the body and the mode of arrangement of the axial filament. The body is slender and usually assumes the shape of a club or an elongated spindle, nearly oval in its cross-section: or it may be rather flattened and ribbon-shaped, showing more or less distinct edges at both sides. The body is twisted and spirally wound in a characteristic manner, showing two to five undulations as seen from the side (commonly two or three, but four or five in some species). The body is less metabolic than in *Pyrsonympha*, though abnormally thickened and shortened forms are met with rarely in some species. Such individuals may resemble small forms of *Pyrsonympha*, but are easily distinguished from the latter by the characteristic feature of the axial filament.

The degree of development of the axial filament varies in different species. In some it is thick and distinct, while in others it is quite slender, indistinct, and even almost unnoticeable in some species. In some forms it is almost uniform in thickness throughout its entire length; in others it gradually diminishes in thickness towards the posterior end, while yet in others it is indistinct in only the middle portion. The filament does not hang freely in the endoplasm, but its posterior end is fixed at the posterior extremity of the body. The axial filament is, moreover, usually adherent to the body wall for the greater part of its length, and it is not capable—as in *Pyrsonympha*—of independent lashing movements in the cytoplasm. In two species (*D. leidyi*

and *D. parva*) it is very indistinct and hardly visible in the majority of individuals, except at the anterior end—probably owing to its slenderness.

Just as in *Pyrsonympha*, the anterior tip of the axial filament is situated at the anterior tip of the body, and the flagellar cords arise at this point. In well-developed axial filaments, the anterior end is often slightly thickened, and two parts are then distinguishable at this end; namely, a main central portion, which is faintly stained, and a peripheral zone which stains intensely.

The mode of arrangement of the flagellar cords on the surface of the body differs from that in *Pyrsonympha*. The cords run parallel with the longitudinal axis of the body, and their distribution is not uniform over the entire surface, but is limited to one-half of the surface or a little more. It is on the surface occupying the outside in the spirally curled body that the flagellar cords are found, the surface occupying the inside being destitute of them. The flagellar cords are straight in their course, those of only one species, viz. *D. rugosa*, forming an exception, where they are fairly wavy. In the majority of the species, each cord is situated on a distinct ridge, so that the outline of the organism appears beautifully serrated; but in a few species the ridges are absent or very indistinct, and the surface appears entirely or almost smooth. The surface destitute of cords is smooth in all species. The free portions of the flagellar cords at the posterior end of the body commonly measure from one-fifth to one-fourth of the body length, except in one species (*D. leidyi*) where they are conspicuously long.

In some species the body is entirely naked, while in many others peculiar filamentous appendages, such as are seen in *Holomastigotoides* and *Pyrsonympha*, are found on it. In some forms these appendages are present in large numbers, and are distributed over the greater portion of the surface of the body, while in others they are limited to a small area. Their mode of distribution on the body is rather regular, and in one form they are arranged in very regular transverse rings. The nature of these filamentous structures will be considered afterwards.

The nucleus is situated at the anterior end, except in one species (*D. rugosa*), in which it is found in the middle portion of the body. In one other species (*D. exilis*) individuals with a terminal nucleus and those with it separated from the anterior end are both common. I am not sure whether or not such individuals are ever to be seen in the other species. The internal structure of the nucleus is of the same type as that of *Pyrsonympha*. When situated at the anterior end of the body, it is oval or piriform. In the majority of forms its posterior end is rounded, whilst it narrows towards the anterior pole. The narrowed end of the nucleus is usually found at the extreme anterior end of the body, where the anterior tip of the axial filament is also fixed. From this disposition, it cannot be doubted that the nucleus and the axial filament are kept in connexion with each other, just as in the forms of *Pyrsonympha*. The nucleus when localized at the middle region appears commonly round, but one peaked point is often distinctly noticeable (Pl. XV, fig. 72) which may be taken

as an indication of the existence of a connexion between the nucleus and some other part of the body—perhaps the axial filament, as in the other forms. As mentioned already, the American authors did not describe any connexion between the nucleus and the axial filament in the *Pyrsonympha* studied by them; but the shape and the position of the nucleus, as illustrated by them, show resemblances to the conditions in some of the species of *Dinenympha* studied by myself, and I am of the opinion that a special structure connecting the nucleus and the axial filament exists also in the American species of *Pyrsonympha*.

Like *Pyrsonympha*, the species of *Dinenympha* live both hanging from the intestinal wall and swimming freely in the lumen of the gut. Freely swimming individuals move very vigorously. They display both rotation and flexion, and in slender forms the anterior end is actively swung in all directions.

Dividing forms are met with very rarely. The process of division seems, as far as I could conjecture from several stages that came under my notice, to be of the same type as that observed in *Pyrsonympha* (Pl. XV, figs. 83 and 84).

Several forms are to be distinguished in this subgenus, and in my earlier account (1917) I described them as nine species and one variety. At present, however, I feel that it is better to regard the independence of some of the forms as questionable, and here I propose to classify them under six species and one doubtful species.

The forms harboured by *Leucotermes speratus* of Japan proper and those in *Leucotermes flaviceps* of Formosa are not identical, three species and the doubtful one being common to both species of termite.

(1) *Dinenympha exilis* sp. nov. (Plate XV, figs. 67 and 68, and Text-fig. E, *a*).

The body is long and slender, the greater portion being almost uniform in thickness though it tapers gradually at the extremities. It measures 50–100 μ in length and 4–8 μ in width at the middle. The number of complete spiral turns of the whole body is commonly two or two and a half: so that in side view four or five undulations are commonly visible. When the organism does not move very actively, its body is oval or elliptical in cross section; but when it moves actively the body becomes somewhat more flattened. The surface of the body is almost smooth, the ridges on which the flagellar cords are situated being very indistinct. The axial filament is thick, but becomes slightly thinner towards the posterior end. The endoplasm is clear and food débris is rarely contained in it. The nucleus is of an elongated oval shape, and is situated at the anterior extremity of the body, as a rule; but occasionally it is found near the middle or in the hinder regions.

This species is found in both *Leucotermes speratus* and *L. flaviceps*. No forms taken to be of this type are found in the descriptions of the American authors.

(2) *Dinenympha rugosa* sp. nov. (Plate XV, figs. 69–72, and Text-fig. E, *b*).

The body is long and slender as in the preceding species; but it is flat and ribbon-like, both sides being rather distinctly edged, and it tapers more abruptly at the posterior end. The length is almost equal to that of *D. exilis* but the width is about twice as great. The surface is not smooth but is provided with high and distinct ridges, and the flagellar cords are markedly wavy. In young individuals with only four cords the ridges are particularly prominent. The axial filament is slender in this species, in contrast with *D. exilis*, being slightly thicker than the flagellar cords at the anterior portion and nearly equal to them at the hinder end, so that it is here hardly distinguishable from them. The endoplasm appears remarkably coarse and is frequently vacuolated, showing an appearance quite different from that of *D. exilis*. The nucleus is situated near the middle of the body. It is commonly round in shape, but in some forms a peaked point is noticed, as shown in Pl. XV, fig. 72, which may be taken as an indication of the existence of a connexion between the nucleus and a certain portion of the body.

The species is harboured by *Leucotermes speratus* only. Forms of this type appear to be lacking in the descriptions of American authors.

(3) *Dinenympha nobilis* sp. nov. (Plate XV, figs. 73–75, and Text-fig. E, *c*).

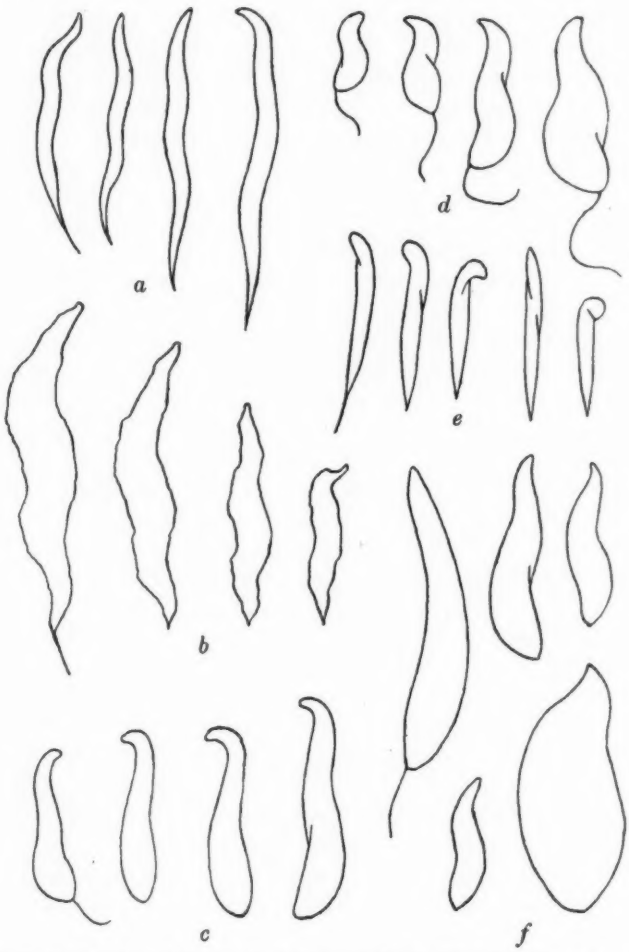
The body assumes a club shape, gradually thickened towards the posterior end, and measures 30–60 μ in length and 5–10 μ in width. It is almost evenly rounded at both ends and is oval in cross section, the surface being quite smooth. Twisting of the body is slight, and the anterior portion is usually turned back, so that it assumes commonly the shape of a hook. The axial filament is rather indistinct. It is fairly thick anteriorly and becomes gradually slender towards the middle, where it is usually almost invisible; but it then becomes gradually thicker again posteriorly, and the extreme end is specially thickened. The endoplasm is of a peculiar appearance in this species: it is dark and finely granular during life, usually containing plenty of food débris.

This species seems lacking in the Formosan species of termite, having been found in *Leucotermes speratus* only. No forms of this type seem to have been illustrated by the American authors.

(4) *Dinenympha leidyi* sp. nov. (Plate XV, figs. 76 and 77, and Text-fig. E, *d*).

The body is lanceolate and is fairly thick at the posterior portion. The ridges on the surface are very distinct and are arranged regularly. The axial filament is very indistinct, being made out with difficulty at the anterior region, and invisible at the posterior. The most remarkable feature of this form is that the free ends of the flagellar cords are conspicuously long, reaching

a length equal to about one-half that of the body. Filamentous appendages are found usually distributed thickly over a limited area of the surface, free from the flagellar cords, at the posterior end of the body, and small numbers of them are also found on the other parts of the body. The length of the



Text-fig. E. Outline drawings of the species of *Dinenympha*. Drawn with camera lucida. ($2 \times \frac{1}{12}$.)
a, D. exilis; b, D. rugosa; c, D. nobilis; d, D. leidyi; e, D. parva; f, D. porteri.

organism is usually between 25 μ and 50 μ, the width some 8 μ to 15 μ. The nucleus is spherical or oval, and situated at the anterior extremity.

This form is found in common in both the Japanese species of *Leucotermes*. The forms described by Leidy under the name of *Dinenympha gracilis* appear

to be similar, but this latter species is distinguishable by its greater length and more tapering posterior end; and apparently its free flagella are shorter, judging from the figures of Leidy and Porter.

(5) *Dinenympha parva* sp. nov. (Plate XV, fig. 78, and Text-fig. E, *e*).

This is the smallest member of the whole group, measuring 20–45 μ in length and 3–5 μ in width. The body is slender and appears almost straight, excepting the anterior portion, which is often curved or bent back. It appears simply twisted, but it is also wound slightly in a spiral in many individuals. The ridges on which the flagellar cords are situated are very indistinct, and the surface of the body is almost smooth. The nucleus is spherical and is situated at the anterior extremity of the body. The axial filament is very slender and indistinct, being made out only in exceptional cases, and is found at the anterior end only. The endoplasm has a characteristic appearance, containing commonly a large quantity of globular bodies taking iron-haematoxylin rather deeply. The peculiar filamentous appendages are found in small numbers on the posterior portion, and sometimes also at the anterior end.

This species occurs in *Leucotermes speratus* and *L. flaviceps*. No forms closely resembling it have ever been described by previous authors.

(6) *Dinenympha porteri* sp. nov. (?) (Plate XV, figs. 79–82, and Text-fig. E, *f*).

The body assumes a fusiform or lanceolate shape, pointed at both ends or rather rounded at the posterior end. It measures 25–80 μ in length and 6–15 μ in width. In some forms the body is somewhat flat and its edges are rather distinct, while in others the body is fairly thick and no distinct edges are recognizable. The axial filament is thick and distinct throughout its entire length, and a rhombic enlargement of it can be recognized at the posterior end in some specimens (Pl. XV, fig. 82). Some individuals are entirely naked, as in the preceding species, while in others the filamentous appendages are found only on certain portions, or over almost the entire surface of the body. As regards the coat of filamentous appendages, and the other characters, the following four types are distinguishable.

Type 1. The body is lanceolate and edged at both sides, 30–60 μ in length and 7–15 μ in width. No filamentous appendages are present (Pl. XV, fig. 79).

Type 2. The shape and size of the body as in the forms of the preceding type. A group of filamentous appendages, varying from about 12 to 24 in number, is found at the posterior end of the body. At the anterior end, moreover, one or two pairs of filamentous appendages are found. These filamentous structures are commonly longer and thicker than those at the posterior end of the body: they differ in appearance from the latter, and in some individuals their attachment to the anterior end of the axial filament can be made out (Pl. XV, fig. 80).

Type 3. The body is lanceolate or club-shaped, and its edges may be distinct or indistinct. Commonly the organism is longer and more slender than those of the above two types, measuring 25–80 μ in length and 6–10 μ in width. The axial filament is very thick. The body is thickly covered with filamentous appendages, diffusely distributed over almost the entire surface. At the posterior region they are particularly dense, and at the anterior end they are somewhat denser than in the middle portion (Pl. XV, fig. 81).

Type 4. Resembles the forms of the foregoing type, and is usually indistinguishable in fixed and stained preparations. The mode of arrangement of the filamentous appendages, however, is very peculiar in this type. They are not distributed diffusely on the surface but are arranged in transverse rows varying from 12 to 18 in number. The body is a little smaller than in the preceding type.

Whether any or all of these types should be regarded as distinct species or not is a question hard to answer decidedly at present. To give a correct answer the nature of the filamentous appendages must be exactly determined. My observations in this respect being still inadequate, my knowledge of these structures is limited. Some of the filaments, such as those seen at the posterior end of the body of the forms of Type 2, are probably micro-organisms attached to the body wall. Some of them, however, such as those at the anterior end of the forms of the same type, seem to have certain peculiarities, if taken for attached micro-organisms. As for those such as are seen in Type 4, I am of the opinion that they should not be regarded as micro-organisms, but are rather similar to, or homologous with, the "bristles" described by Janicki (1915) in *Parajoenia*. In my former paper in Japanese I described each of these types under a specific name (*D. nuda*, *D. corniculata*, *D. porteri*, and *D. comosa* respectively); but now I think it better to leave the question of their identity in doubt, provisionally calling the group by the name *D. porteri*.

PART II. GENERAL DISCUSSION.

1. COMPARATIVE MORPHOLOGY.

The forms belonging to the *Trichonympha* series are of very varied organization, and the differences among them are so striking in many cases that one cannot help doubting whether they belong to one and the same group in the system. Both the forms of complicated organization, as well as those of rather simpler structure, are provided with some peculiarities. The members of the *Pyrsonympha* series also have many peculiarities in their organization. Consequently, the comparative morphological study of all these forms is most interesting and important. Notwithstanding, however, a rather rapid advance in our knowledge in recent years, this subject has hardly been touched upon by any authors: but as I have fortunately been able to study many forms and, especially, the process of division in several of them, I shall undertake to discuss these questions here.

Let us begin with the comparative study of the most complicated and most peculiar organs in *Trichonympha*, *Teratonympha*, and *Pseudotrichonympha*; namely, the structures in the nipple and those in connexion with the nucleus. It will be helpful to give here a brief review of the structures which I am going to consider. In *Trichonympha*, there is an *axial core* consisting of two parts, viz. the wall and its contents; the nucleus is held at the bottom of a *corbule*; and there is also a *columnar body* hanging perpendicularly from the base of the axial core to the anterior pole of the nucleus. In *Teratonympha*, the *axial core* is a solid cone, but is surrounded by a special layer of protoplasm, filling up the space between it and the *tubular rind*; the nucleus is enveloped in a peculiar *nuclear sac*, which is closely connected with the body wall and the above-mentioned layer by means of its flange and its neck respectively. I conjecture that the protoplasm around the core is of the same substance as that of the nuclear sac. I conjecture also that the contents of the axial core of *Trichonympha* is homologous with the layer around the axial core in *Teratonympha*, and that the nuclear sac of *Teratonympha* is also homologous with the corbule and the columnar body in *Trichonympha*. The contents of the axial core of *Trichonympha* is granular, but the layer around the axial core of *Teratonympha* is homogeneous. Thus it may seem unreasonable to take the two to be of the same substance; but I think the contents of the axial core of *Trichonympha* consists really of two substances, viz. a homogeneous ground substance and granules embedded in it—the former being comparable with the protoplasm around the axial core in *Teratonympha*, while the granules are lacking in the latter. Thus I interpret these two genera to show a rather distinct similarity in organization, as regards the points discussed above; the differences being the relative position of the two components in the nipple, and the greater differentiation of the axial core in *Teratonympha*.

Resemblances between *Trichonympha* and *Pseudotrichonympha* are distinct and easily recognizable. The *axial core* of *Pseudotrichonympha* is clearly comparable with that of *Trichonympha*. The structures in *Pseudotrichonympha*, consisting of a *tubule* and a *ball*, may be safely taken to represent a type of structure quite similar to that of *Teratonympha*, and they may also be taken to show a further differentiation of the axial core, with its column and knob-like end, of *Trichonympha*. A difference, however, is recognizable between *Trichonympha* and *Pseudotrichonympha*: for neither a corbule, nor any structure corresponding to it, is distinguishable in *Pseudotrichonympha*.

As regards the rôle played by the axial core of *Trichonympha* during the process of division, the observations of the Italian and American authors agree in essential points with my own. In this genus, the mitotic figures are not formed from the nucleus only, but the strand ("fuso esterno" of Foà, "paradesmose" of Kofoid and Swezy) and the division-centre are of extranuclear origin, being furnished by the nipple. Foà, and Kofoid and Swezy, agree in the view that the axial core plays the part of a division-

centre in mitosis. I also failed to find any structures such as centrosomes at the ends of the strand, and I agree with the above view. As for the origin of the strand, I think it is probably derived from the wall of the axial core.

In *Teratonympha* and *Pseudotrichonympha* the condition is somewhat different. Here the axial core does not function as a division-centre, but a special structure is formed for the purpose. In the early stages of division of *Teratonympha* a strand is seen, quite independent from the nucleus, provided with a "central body" at both its ends, each of these being connected with the base of the daughter nipples by means of a thread. As regards the origin of these structures, I think they are derived from the axial core. Thus I believe that the axial core of both *Teratonympha* and *Trichonympha* is the source of the structures of extranuclear origin in mitosis; but these organisms differ in one point, namely, in *Teratonympha* the axial core gives rise to the body functioning as a centrosome, whilst in *Trichonympha* it acts itself as a division-centre. In *Pseudotrichonympha* the condition is quite similar to that of *Teratonympha*: namely, the spherical body and the thread connecting it with the nipple are derived from the tubular column, and probably, I think, from its wall, whilst the strand arises from the spherical body.

The homology of the two layers at the anterior region of *Trichonympha*, *Teratonympha*, and *Pseudotrichonympha* is readily recognizable; the differences being simply in degree of development. The axial core in *Trichonympha* is surrounded concentrically by the inner and outer layers, the basal granules of the flagella being localized at the basal or proximal surface of the outer layer. In *Pseudotrichonympha* almost the entire surface of the axial core is directly surrounded by the outer layer, and only a small portion of its posterior part is in contact with the inner layer; consequently the basal granules, in this region, are localized immediately on the surface of the axial core. In the bell, the distribution of the layers is identical in both genera. In *Teratonympha* the basal granules of the flagella are found at the base of the outer layer, just as in the other genera, and the conical axial core is in contact with the inner layer (tubular rind) at its anterior end. As they are partly separated by the layer of peculiarly differentiated protoplasm, the axial core and the inner layer of *Teratonympha* seem not so intimately connected as in the other genera. But it is not only at the anterior end that these two parts are connected: there is also the vertical partition crossing the space between them, and keeping them in close connexion. Thus the inner layer seems to serve as a means of connexion between the locomotory organs and the part of the body which plays the rôle of the division-centre in mitosis.

In *Microspironympha* the anterior portion of the body is not so highly differentiated as in the above three genera; but there is a peculiar tubular structure situated in the axis of the body, connecting the tip of the body and the anterior surface of the nucleus. It is easily imaginable that this is a structure corresponding to the axial core in *Trichonympha*. Moreover, it seems not unreasonable to interpret the tubular wall and its contents as

respectively homologous with the wall of the axial core and its contents in *Trichonympha*. My knowledge of the process of division in this species is incomplete, but it seems highly probable that the slender strand and the structures acting as division-centres at both its ends are derived from the tubule. Each row of basal granules starts at the tip of the tubule, and the relation between them seems also analogous with what is seen in *Trichonympha*. There is, moreover, a structure which I conjecture to be homologous with a nuclear sac or corbule. This is the special protoplasm enveloping the tubule and the nucleus. The outer boundary of this layer of protoplasm is not distinctly contoured, but it is especially distinct at the base of the tubule and the anterior pole of the nucleus. As described in a previous section, the posterior end of the tubule is commonly found fixed on the nuclear wall; but sometimes they are separated from each other and connected by means of a mass of dense protoplasm, in which the contents of the tubule and the special protoplasm around it are not distinguishable. Thus I think there are many reasons indicating that the dense protoplasm and the contents of the tubule are composed of the same substance; and, moreover, that they are homologous with the nuclear sac, the corbule, and the contents of the axial core. From the above data, it may be concluded that the structure of *Microspironympha* is similar to that of *Trichonympha*, but of a less complex type.

In *Holomastigotoides*, the organization of the anterior portion is apparently of a quite different type. There is no structure corresponding to the tubule in *Microspironympha* or the axial core in *Trichonympha*. All parts of the mitotic figure seem to be formed from the nucleus itself, and both the division-centre and the strand appear on the nucleus: so that it is reasonable to suppose that a body which acts as a division-centre is localized on the nuclear wall. The most interesting structure of *Holomastigotoides*, from the viewpoint of comparative morphology, is the peculiar mass of special protoplasm surrounding the nucleus and occupying the area in front of it. It is clearly observable in good preparations that the anterior tip of this mass reaches the anterior extremity of the body, where the ends of the rows of basal granules of the flagella are kept in connexion with it. That is to say, in this genus the body playing the rôle of the division-centre lies separated from the basal granules of the flagella, but they are kept in connexion by means of the above-mentioned protoplasm. I believe that it is reasonable to interpret this structure as homologous with the dense protoplasm and the contents of the tubule of *Microspironympha*, and consequently with the corbule of *Trichonympha* and the nuclear sac of *Teratonympha*.

In some individuals of *Holomastigotoides* the particular protoplasm is remarkably developed, assuming the form of a distinct and fairly long column hanging along the longitudinal axis of the body. In *Microspironympha* some individuals are found provided with a structure at the posterior portion of the body, appearing sometimes as a well-developed transparent style. I am of the opinion that both these structures are similar in nature,

and that the style in *Microspironympha* is of the same substance as, and most probably continuous with, the contents of the tubule and the layer surrounding the nucleus. Furthermore, I conjecture that these structures in *Microspironympha* and *Holomastigotoides*, together with the nuclear sac in *Teratonympha* and the corbule in *Trichonympha*, are homologous with the axostyle and the structure enveloping the nucleus in *Joenia*, *Devescovina*, *Lophomonas*, and related forms.

As for the other two genera of the *Trichonympha* series which I have studied, viz. *Spirotrichonympha* and *Holomastigotes*, no dividing stages were observed, and our knowledge of these forms is too scanty for discussion. But I believe that these forms represent types similar to, but simpler than, that of *Holomastigotoides*. There is a mass of dense protoplasm connecting the anterior ends of the rows of basal granules with the nucleus; and I suppose that the latter gives rise to all parts of the mitotic figure.

Let us now consider the structure of the members of the *Pyrsonympha* series. The organization of these forms appears quite simple, but it is, in reality, not less peculiar than that of the *Trichonympha* series. I distinguished many species in this group, and have classified them in two subgenera of a single genus, viz. *Pyrsonympha* and *Dinenympha*. All of these species are undoubtedly of the same type in essential points of organization, showing differences only in degree of development or differentiation of some organs. To make this clear, a brief recapitulation of the structure in *Pyrsonympha grandis*—the species showing the characteristic organization most clearly—may be given here. First, there is an *axial filament*, running in the long axis of the organism, and ending anteriorly at the tip of the body; secondly, there are the *flagellar cords* which arise at the anterior tip of this filament and run backwards, attached on the body wall, to the posterior end of the organism; lastly, there is the nucleus, enveloped in the *nuclear sac*, which is kept in connexion with the axial filament and the body wall. Towards the anterior end, the outer wall of the axial filament stains more deeply than the internal part with iron-haematoxylin, although the boundary between the two parts is not distinct and no definite zones are visible. At the anterior extremity, a sharp differentiation into these two constituents is frequently observable. In some individuals, however, a small portion of the anterior end appears to consist of an axis of feebly stainable substance with deeply stainable cords of almost uniform thickness regularly arranged along its surface. Thus it seems certain that the axial filament consists of two components, which are distinctly differentiated only at the anterior end. In small individuals of *Pyrsonympha grandis*, the axial filament is commonly homogeneous throughout its entire length. In the other species of smaller size, and in the species of *Dinenympha*, the axial filament also commonly appears homogeneous throughout its entire length. In some individuals, however, differentiation into an outer wall, staining intensely with iron-haematoxylin, and a feebly staining centra part, may be observed on rare occasions. The most important feature in the

organization of *Pyrsonympha* is the apparent absence of any basal granules for the flagellar cords. From the stainability of the axial filament and its mode of connexion with the flagellar cords, I think it not unreasonable to conjecture that this organ itself functions as a basal granule or blepharoplast for the flagellar cords, and I would regard the component taking iron-haematoxylin deeply as specially exercising this function.

In my opinion the axostyle of trichomonad flagellates is not homologous with the axial filament of *Pyrsonympha*. The axial filament is not simply a skeletal organ like the axostyle, but an organ playing the rôles of several different structures in the trichomonads, viz. the axostyle and the basal granules.

As the nucleus of *Pyrsonympha* divides, the axial filament becomes detached from it, and the centrosomes make their appearance on the nuclear wall, with half the number of flagellar cords starting from each of them. If my interpretation given above—attributing the nature of a basal granule to the axial filament—be accepted, it may be deductively conjectured that the filament supplies the bodies which play the part of division-centres. In this respect, the axial filament may be taken to be homologous with the axial core in *Trichonympha* and the corresponding structures in other forms of the *Trichonympha* series. The nuclear sac in *Pyrsonympha* seems homologous with the nuclear sac in *Teratonympha* and the corbule in *Trichonympha*, on account of its relations to the axial filament, the body wall, and the nucleus. I am inclined, moreover, to suppose that the part of the filament which does not stain with haematoxylin consists of the same material as the contents of the nuclear sac.

2. CLASSIFICATION.

A great many types are represented by the protozoal organisms harboured by termites. Not only do they differ from each other, but the great majority of them display so many peculiarities that a large number of genera had necessarily to be established for them. At present 38 generic names are recorded in the literature (excluding spirochaetes and gregarines), and I have added two more in this paper. The greater part of this large number of genera, namely 36, consists of those established for organisms found in the intestines of termites exclusively. Among these 36 names, at least three are to be taken as synonyms of others; and in a few of the remainder the descriptions are not sufficient to enable us to understand their characteristics distinctly. A synopsis of the genera recorded from termites has already been given on p. 243.

The organisms peculiar to the termites may be divided into two distinct groups; namely, those provided with numerous or abundant flagella, and those with a small number of these structures. Our "Trichonympha series" comprises several types of the former group, while the "Pyrsonympha series" contains several organisms representing only a single type of the latter.

The first serious attempts to classify these organisms, and to arrange them in a system, were made by Grassi (Grassi, 1892; Grassi and Sandias, 1893; Grassi [and Foà], 1911). In 1911 he established a new order, Hypermastigina, in the Flagellata, for the group of organisms provided with numerous flagella, and suggested that three families might be distinguished. These families were not named, however. The first would include *Eulophomonas*, *Lophomonas*, *Mesojoenia*, *Joenia*; the second *Trichonympha*; the third *Spirotrichonympha*, *Holomastigotes*, and *Holomastigotoides*. But Grassi remarks that *Microjoenia* and *Pseudotrichonympha*—which do not fit into this system—make it difficult to arrange the organisms in question in groups higher than genera. The forms with a small number of flagella he classified among the Polymastigina, and established two new families, viz. Calonymphidae and Dinenymphidae, for them.

After Grassi, França (in a paper sent to the editor in 1914 but not published till 1916) and Janicki (1915) published their views on the classification. França divided the Hypermastigina into four families, giving family rank to the genus *Lophomonas*; his four families being Calonymphidae, Lophomonadidae, Trichonymphidae, and Holomastigotidae. Janicki proposed, following Grassi, to classify the Calonymphidae among the Polymastigina, and to divide the Hypermastigina into the following four families: Lophomonadidae, Joenidae, Trichonymphidae, and Holomastigotidae. In his most recent paper, Grassi (1917) has described a large number of forms which he classifies in eight families, belonging to two different orders. In the Hypermastigina he distinguishes five families, viz. Joenidae, Staurojoenidae, Trichonymphidae, Spirotrichonymphidae, and Holomastigotidae. The rest of the forms, which he assigns to the Polymastigina, are classified in the three families Tetramitidae, Dinenymphidae, and Calonymphidae.

As many of the organisms are still not thoroughly investigated, their classification can but be, at present, provisional, and alterations and improvements will inevitably accompany progress in our knowledge. The forms studied by myself belong to more than a few types, but not to so many as to enable me to attempt a final classification of the entire group of these organisms. Accordingly, it is rather my intention in this section to consider the classification of those forms only which I have myself studied, and to make clear the characters of the genera and families to which these forms belong.

Trichonympha and *Pseudotrichonympha* are closely similar, and it is reasonable to place them near one another in the system. *Teratonympha*, as remarked in the preceding section, shows a rather close resemblance, in the structure of the anterior portion of its body, to *Trichonympha* and *Pseudotrichonympha*. *Teratonympha*, however, displays some distinct peculiarities in both the anterior and posterior regions of its body; and I think these sufficient to justify me in establishing a new family, which I propose to call Teratonymphidae, for these organisms.

In the system of Grassi, *Holomastigotes* and *Holomastigotoides* are classified separately from *Spirotrichonympha*. The differences between these genera, however, do not seem to me so remarkable as to warrant their separation into different families. The peculiarly differentiated protoplasm in the anterior region of the body is similar in the three genera, differing simply in the degree of its development. The organisms agree also in the character that the flagella are arranged in spiral rows: and in all these forms, also, the spiral arrangement is peculiar in being dexiotropic. *Spirotrichonympha* seems to differ from the other genera in that the basal granules of the flagella lie somewhat deep in the endoplasm; but I do not think this difference to be very important. As for my *Microspironympha*, it has the rows of flagella running spirally (dexiotropic), just as in the above three genera; but it differs from them in being provided with the tubular structure connecting the anterior tip of the body with the nucleus. The differences between the Holomastigotidae and Spirotrichonymphidae of Grassi are not so distinct as the differences between *Microspironympha* and any of the Spirotrichonymphidae and Holomastigotidae. Consequently if *Holomastigotes* and *Spirotrichonympha* are taken to belong to different families, *Microspironympha* would naturally have to be separated from both of them. But I do not think the differences among *Microspironympha*, *Holomastigotes*, and *Spirotrichonympha* are so important that each must be taken to represent the type of a different family. I am of the opinion that these genera should all be put at present in a single family, which may be called Holomastigotidae.

The forms provided with a smaller number of flagella are classified among the Polymastigina by Grassi. They are of very varied types and the great majority of them have their own peculiarities: so I suppose that, as our knowledge increases it will ultimately be necessary to establish many families for them. For instance, some of the forms classified by Grassi among the Tetramitidae will perhaps soon be separated from the ordinary trichomonads. In this group, my material was limited to only one genus, *Pyrsonympha*. *Pyrsonympha* has very distinct characteristics, and it is unquestionable that it has to be separated from any families established for flagellates other than those from the intestines of termites. I cannot, indeed, find any form among hitherto described flagellates which seems to belong to the same family: for I cannot agree with Grassi (1917) in ranking *Pseudotrypanosoma*, described by him, in the same family with *Pyrsonympha*.

The families and the genera studied by myself will be defined as follows:

(1) Family *Trichonymphidae* S. Kent (emend.).

Large forms. Anterior part of the body highly differentiated: body wall of the anterior portion formed of one or two thick ectoplasmic layers, densely traversed by numerous flagella: anterior extremity provided with an axial skeleton (axial core). A single large vesicular nucleus present. There is no

permanent mouth, food being ingested through the general surface of the hind end of the body. A peculiar differentiation of the protoplasm surrounding the nucleus is present in the anterior region. It may assume the form of a hemispherical bowl (corbule) and a columnar mass; or it may be distributed diffusely, or merely form a layer round the nucleus. The rest of the body not distinctly ridged; naked or flagellated. Flagella arranged in longitudinal rows at anterior end of the body, and sometimes restricted to this region. Sometimes, however, the rows extend over the greater part of the body, and in this case they are arranged in spiral lines (laeotropic) in the posterior region. Multiplication by simple longitudinal fission, the nucleus dividing by mitosis. No cysts or sexual stages known.

Genus I. *Trichonympha* Leidy, 1877.

Anterior part of the body distinguishable into two parts, the nipple and the bell. The nipple consists of an axial core with two layers surrounding it. The wall of the bell also consists of two similar layers. A corbule and a columnar mass of peculiar protoplasm are present, the nucleus being kept at the bottom of the former and at the base of the latter. Flagella arising in longitudinal rows from nipple and bell. The hinder part of the body, posterior to the bell, free from flagella[1]. Type species *T. agilis* Leidy, 1877. (In *Leucotermes* (*Reticulitermes*) *flavipes*, N. America.)

Genus II. *Pseudotrichonympha* Grassi, 1911.

Anterior part of the body distinguishable into two parts, as in *Trichonympha*. The axial core consists of a column with a ball at the tip, and is surrounded by a single layer of ectoplasm. The wall of the bell consists of two layers. The nucleus lies freely in the endoplasm. Body posterior to bell furnished with spiral rows (laeotropic) of short flagella, excepting a small area at the posterior extremity. Type species *Ps. hertwigi* (Hartmann) Grassi, 1911. (In *Coptotermes hartmanni*, Brazil.)

(2) Family *Teratonymphidae* (fam. nov.).

Large forms. Anterior end of the body highly differentiated as in the Trichonymphidae, but more complex in structure. Body regularly ridged transversely, so that it appears metamerically segmented. Each ridge provided with a single row of flagella. No visible mouth. The single vesicular nucleus is enclosed in a nuclear sac, fixed to the body wall and base of the head, and lies in the first apparent segment of the body. Multiplication by simple longitudinal division, with mitosis of nucleus. No cysts or sexual development known. Genus *Teratonympha* (gen. nov.) with characters of family. Type species *T. mirabilis* mihi. (In *Leucotermes* (*Reticulitermes*) *speratus*, Japan.)

[1] If the description of Kofoid and Swezy (1919) is right, I do not take their *Trichonympha campanula* to belong to this genus. *Leidyopsis* described by them appears to be closely similar to (probably synonymous with) *Gymnonympha* of Dobell, and I think both belong to this family.

(3) Family *Holomastigotidae* Grassi (emend.).

Body not distinctly divided into different parts—as in *Trichonympha*. Flagella arranged in spiral rows from their commencement at anterior end; direction of spiral dexiotropic. A part of the hind end of the body—which may be large or small—free from flagella. A peculiar mass of dense protoplasm exists at the anterior portion of the body, and surrounds the nucleus, but in some genera this mass is less dense in the immediate vicinity of the nucleus. A tubular organ in connexion with this structure may or may not be present. Surface of the body ridged or apparently smooth. Nucleus single, vesicular. No visible mouth. Multiplication by simple longitudinal division, with mitosis of nucleus. No cysts or sexual stages known.

Genus I. *Holomastigotes* Grassi, 1892.

Small forms. Surface of the body with spiral ridges, few in number. Basal granules of flagella situated at the bottom of the groove behind each ridge, the basal parts of the flagella being fixed on the surface of the ridges. A dense mass of protoplasm is present at the anterior end, the nucleus being embedded in it. Nucleus anterior, spherical. Type species *H. elongatum* Grassi, 1892. (In *Leucotermes* (*Reticulitermes*) *lucifugus*, Italy.)

Genus II. *Holomastigotoides* Grassi, 1911.

Large forms. Surface of the body with numerous spiral ridges, the flagella being attached as in the preceding genus. A mass of dense protoplasm is present at the anterior portion, and distinctly developed: it surrounds and sometimes extends behind the nucleus. Nucleus anterior, ovoid or compressed antero-posteriorly. Type species *H. hertwigi* (Hartmann) Grassi, 1911. (In *Coptotermes hartmanni*, Brazil.)

Genus III. *Spirotrichonympha* Grassi, 1911.

Medium-sized forms. The rows of basal granules of the flagella lie somewhat more deeply in the endoplasm than in the preceding genera; being deepest at the anterior end and becoming more superficial towards the posterior. A fairly large part of the root of each flagellum is thus embedded in the protoplasm. The mass of dense protoplasm assumes the shape of a cone, its hinder border being indistinct. The spherical nucleus lies apparently free in the endoplasm, at some distance from the anterior extremity. Type species *Sp. flagellata* Grassi, 1892. (In *Leucotermes* (*Reticulitermes*) *lucifugus*, Italy.)

Genus IV. *Microspironympha* (gen. nov.).

Small forms. Surface of the body not ridged: the rows of basal granules lie under the surface, but are somewhat more deeply placed at the anterior end. Nucleus spherical, anterior, but separated from the anterior extremity

by a tubular structure, connecting its anterior pole with the anterior tip of the body. The spiral rows of flagella arise from the anterior end of this structure. A mass of denser protoplasm is also present surrounding the tubule and the nucleus. An axial rod sometimes (? always) present. Type species *M. porteri* mihi. (In *Leucotermes* (*Reticulitermes*) *speratus*, Japan.)

The three foregoing families belong to Grassi's order Hypermastigina. The remaining family contains organisms which differ considerably from all those just considered.

(4) Family *Pyrsonymphidae* Grassi.

Large or small forms. An elastic thread (the axial filament), of variable thickness, runs longitudinally down the body. From the anterior tip of the body a small number of flagellar cords start at the anterior extremity of this filament, and run spirally backwards attached on the surface of the body, becoming free as flagella at the posterior end. Spiral direction of cords laeotropic in all species studied. Nucleus single; variable in shape but usually anterior or central in position.

Genus *Pyrsonympha* Leidy, 1877 (*emend.*).

Body piriform, club-shaped, spindle-shaped, or screw-like: simply twisted, or both twisted and spirally wound. Number of flagellar cords 4 or 8.

Subgenus I. *Pyrsonympha* Leidy (*emend.*).

Body piriform, club-shaped, or screw-like; spirally twisted. The axial filament hangs in the endoplasm, its posterior end being free from the body wall. Type *P. vertens* Leidy, 1877. (In *Leucotermes* (*Reticulitermes*) *flavipes*, N. America.)

Subgenus II. *Dinenympha* Leidy (*emend.*).

Body slender and wound spirally, sometimes also spirally twisted. Axial filament ends fixed at the posterior tip of the body, or is indistinct except at the anterior end. Type *D. gracilis* Leidy, 1877. (In *Leucotermes* (*Reticulitermes*) *flavipes*, N. America.)

As regards the systematic position of the Pyrsonymphidae I am still in doubt. Most recent authors—including Grassi—place this family among the Polymastigina: but the organisms appear to me so different in many ways from all the other forms in this order, that I hesitate to assign them to this group. It seems to me that it will be necessary to found a new order to contain them; but as the classification of the whole order Polymastigina is still defective, owing to our incomplete knowledge of many forms, I shall not propose a name for this suggested new order at present.

BIBLIOGRAPHY.

BUGNION, E. (1912). Observations sur les Termites. Différenciation des castes. *C. R. Soc. Biol.* LXXII. 1091.

—— (1913). La différenciation des castes chez les Termites [Nevr.]. *Bull. Soc. Entomol. France*, p. 213.

BUGNION, E. and FERRIÈRE, C. (1911). L'imago du *Coptotermes flavus*. Larves portant des rudiments d'ailes prothoraciques. *Mém. Soc. Zool. France*, XXIV. 97.

BUGNION, E. and POPOFF, N. (1910). Le termite à latex de Ceylan, *Coptotermes travians* Haviland. *Ibid.* XXIII. 105.

—— (1910 *a*). Les *Calotermes* de Ceylan. *Ibid.* XXIII. 124.

BUSCALIONI, L. and COMES, S. (1910). La digestione delle membrane vegetali per opera dei Flagellati contenuti nell' intestino dei Termitidi e il problema della simbiosi. *Atti Accad. Gioenia Sci. Nat. Catania*, Ser. 5, Vol. III. *Mem.* XVII. 1.

BUTTEL-REEPEN, H. v. (1914). Wissenschaftliche Ergebnisse einer Forschungsreise nach Ostasien. III. Termiten aus Sumatra, Java, Malacca und Ceylon [von NILS HOLMGREN]. Vorwort. *Zool. Jahrb.* (*Syst. Abt.*) XXXVI. 229.

COMES, S. (1910). *Lophophora vacuolata* (Comes) nuovo genere e nuova specie di flagellato dell' intestino dei Termitidi. *Boll. Accad. Gioenia Sci. Nat. Catania*, (Ser. 2) Fasc. 13, p. 11.

—— (1910 *a*). Alcune considerazioni citologiche a proposito del dimorfismo sessuale riscontrato in *Dinenympha gracilis* Leidy. (Nota prelim.) *Ibid.* p. 20.

—— (1912). Fenomeni nucleari e fasi riproduttive in *Pyrsonympha flagellata* Grassi. (Nota prelim.) *Ibid.* (Ser. 2) Fasc. 20 and 21, p. 6.

—— (1912 *a*). Riproduzione e morfologia di *Dinenympha gracilis* Leidy flagellato ospite dell' intestino dei Termitidi. *Arch. f. Protistenk.* XXV. 275.

—— (1914). Notizie sulla morfologia e riproduzione di *Monocercomonas termitis*. *Boll. Accad. Gioenia Sci. Nat. Catania*, (Ser. 2) Fasc. 33, p. 15.

CUTLER, D. W. (1919). Observations on the Protozoa parasitic in the hind gut of *Archotermopsis wroughtoni* Desn. Part I. *Ditrichomonas* (*Trichomonas*) *termitis* Imms. *Quart. Journ. Micro. Sci.* LXIII. 555.

—— (1920). Protozoa parasitic in Termites. Part II. *Joenopsis polytricha* n. gen., n. sp., with brief notes on two new species, *Joenopsis cephalotricha* and *Microjoenia axostylis*. *Ibid.* LXIV. 383.

DOBELL, C. C. (1910). On some parasitic protozoa from Ceylon. *Spolia Zeylanica*, VII. 65.

DOGIEL, V. (1916). Researches on the parasitic protozoa from the intestine of termites. [Russian and English.] I. Tetramitidae. *Journ. russe de Zool.* (Petrograd), I. 1.

FOÀ, A. (1904). Ricerche sulla riproduzione dei Flagellati. II. Processo di divisione delle Triconinfe. (Nota prelim.) *Atti R. Accad. Lincei*, (5 Ser.) *Rendiconti*, XIII. (2 Sem.) p. 618.

—— (1905). Due nuovi Flagellati parassiti. (Nota prelim.) *Ibid.* XIV. (2 Sem.) p. 542.

FRANÇA, C. (1916). Quelques observations sur les Trichonymphidae. *Ann. Inst. Pasteur*, XXX. 195.

—— (1918). Observations sur les Trichonymphides. *Bull. Soc. Portugaise Sci. Nat.* Vol. VIII. pp. 1–10 [separate].

—— (1918 *a*). Observations sur les Trichonymphides. *An. Sci. Fac. Med. Pôrto*, Vol. IV. No. 2 [separate].

—— (1918 *b*). Ma contribution à la connaissance des Trichonymphides. (Réponse à Prof. Battista Grassi.) *Ibid.* Vol. IV. No. 3 [separate].

FRENZEL, J. (1891). *Leidyonella cordubensis* nov. gen., nov. sp., eine neue Trichonymphide. *Arch. f. mikr. Anat.* XXXVIII. 301.

GRASSI, B. (1885). Intorno ad alcuni protozoi parassiti delle Termiti. *Atti Accad. Gioenia Sci. Nat. Catania*, (Ser. 3) XVIII. 235.

—— (1888). Morfologia e sistematica di alcuni protozoi parassiti. *Atti R. Accad. Lincei*, (Ser. 4) *Rendiconti*, IV. (1 Sem.) 5.

—— (1892). Conclusioni d'una memoria sulla società dei Termiti. *Ibid.* (Ser. 5) I. (1 Sem.) 33.

—— (1917) Flagellati viventi nei Termiti. *Mem. R. Accad. Lincei*, (Ser. 5) XII. (fasc. 8) 331.

GRASSI, B. and FOÀ, A. (1904). Ricerche sulla riproduzione dei Flagellati. I. Processo di divisione della Joenie e forme affini. (Nota prelim.) *Atti R. Accad. Lincei*, (Ser. 5) *Rendiconti*, XIII. (2 Sem.) 241.

—— (1911). Intorno ai Protozoi dei Termitidi. (Nota prelim.) *Ibid.* XX. (1 Sem.) 725.

GRASSI, B. and SANDIAS, A. (1893). Costituzione e sviluppo della società dei Termitidi. *Atti Accad. Gioenia Sci. Nat. Catania*, (Ser. 4) Vol. VI. *Mem.* XIII. and Vol. VII. *Mem.* I. (1894). [Also issued separately, Catania, 1893. English translation in *Quart. Journ. Micro. Sci.* Vols. XXXIX. and XL. 1897.]

HARTMANN, M. (1910). Untersuchungen über Bau und Entwicklung der Trichonymphiden (*Trichonympha hertwigi* n. sp.). *Festschr. z. 60ten Geburtstag R. Hertwigs*, I. 351.

HOZAWA, S. (1915)[1]. Revision of the Japanese Termites. *Journ. Coll. Sci. Univ. Tokyo*, Vol. XXXV. Art. 7.

IMMS, A. D. (1919). On the structure and biology of *Archotermopsis*, together with descriptions of new species of intestinal protozoa, and general observations on the Isoptera. *Phil. Trans. Roy. Soc. London*, (B) CCIX. 75.

JANICKI, C. (1910). Untersuchungen an parasitischen Flagellaten. I. *Lophomonas blattarum* Stein, *L. striata* Bütschli. *Zeitschr. f. wiss. Zool.* XCV. 243.

—— (1911). Zur Kenntnis des Parabasalapparats bei parasitischen Flagellaten. *Biol. Centralbl.* XXXI. 321.

—— (1912). Bemerkungen zum Kernteilungsvorgang bei Flagellaten, namentlich bei parasitischen Formen. *Verhandl. Naturforsch. Gesellsch. Basel*, Vol. XXIII. (pp. 1–30, separate).

—— (1915). Untersuchungen an parasitischen Flagellaten. II Teil. Die Gattungen *Devescovina, Parajoenia, Stephanonympha, Calonympha*.—Ueber den Parabasalapparat. —Ueber Kernkonstitution und Kernteilung. *Zeitschr. f. wiss. Zool.* CXII. 573.

KENT, W. S. (1885). Notes on the infusorial parasites of the Tasmanian white ant. *Papers and Proc. Roy. Soc. Tasmania* [for 1884], p. 270. [Reprinted in *Ann. and Mag. Nat. Hist.* (5 Ser.) XV. 450, 1885.]

—— (1880–1882). *A Manual of the Infusoria*. (London.)

KOFOID, C. A. and SWEZY, O. (1919). Studies on the parasites of the Termites. I. On *Streblomastix strix*, a polymastigote flagellate with a linear plasmodial phase. II. On *Trichomitus termitidis*, a polymastigote flagellate with a highly developed neuromotor system. III. On *Trichonympha campanula* sp. nov. IV. On *Leidyopsis sphaerica* gen. nov., sp. nov. *Univ. of California Publ. in Zool.* XX. 1.

KOIDZUMI, M. (1916 and 1917). Studies on the protozoa harboured by the termites of Japan. [In Japanese.] *Report of Investigations on Termites* (*Govt. Inst. of Sci., Formosa*), V. 55 (1916) and VI. 1 (1917).

LEIDY, J. (1877). On intestinal parasites of *Termes flavipes*. *Proc. Acad. Nat. Sci. Philadelphia*, p. 146.

—— (1881). The parasites of the termites. *Journ. Acad. Nat. Sci. Philadelphia*, VIII (N. S.). 425.

[1] A complete list of the papers referring to the termites of Japan is given in this work.

LESPÈS, C. (1856). Recherches sur l'organisation et les mœurs du Termite lucifuge. ***Ann. Sci. Nat., Zool.*** (Ser. 4) v. 227.

PORTER, J. F. (1897). *Trichonympha*, and other parasites of *Termes flavipes*. ***Bull. Mus. Comp. Zool. Harvard College***, XXXI. 47.

SEIP, A. (1881). Parasites of white ants. ***American Monthly Microscop. Journ.*** II. 228.

SIMMONS, W. J. (1890). Parasites of the white ant. ***Ibid.*** XI. 57. [Also French translation in ***Journ. de Microgr.*** XIV. 302 (1890).]

ZULUETA, A. DE (1915). Sobre la reproducción de *Dinenympha gracilis* Leidy. ***Trab. Mus. Nac. Ci. Nat. Madrid***, (Ser. Zool.) No. 23.

DESCRIPTION OF PLATES X–XV.

All the figures, except diagrammatic ones (Figs. 13, 15, 35, 53, 75, 82) were drawn with a camera lucida. The optical apparatus employed was as follows: Zeiss stand IA, immersion objective $\frac{1}{12}$", and Huygenian oculars 2 or 4; tube of microscope not drawn out, and fitted with revolving nosepiece. Drawings made at level of stage of microscope. The dimensions of the organisms are stated in the text. In some figures the surface of the organism is drawn at the lower focus, so that the spirals appear reversed. All figures drawn from specimens fixed with sublimate-alcohol (Schaudinn) and stained with iron-haematoxylin (Heidenhain)—using, however, the haematoxylin in alcoholic solution.

PLATE X.

Trichonympha agilis.

Fig. 1. *T. agilis* var. *japonica*, whole organism. ($4 \times \frac{1}{12}$.)
Figs. 2–4. Anterior portion of *T. agilis* var. *japonica*. ($4 \times \frac{1}{12}$.)
Fig. 5. *T. agilis* var. *formosana*, whole organism. ($2 \times \frac{1}{12}$.)
Figs. 6–12. Stages of division of same. ($4 \times \frac{1}{12}$.)
Fig. 13. Diagrammatic drawing showing structure of the anterior portion of *Trichonympha*. *c*, cap; *a*, axial core—hollow, showing wall and contents, and anterior terminal knob; *i*, inner layer of nipple wall; *o*, outer layer of nipple wall.

PLATE XI.

Pseudotrichonympha grassii.

Fig. 14. Whole organism. ($2 \times \frac{1}{12}$.)
Fig. 15. Diagrammatic drawing of structure of the anterior portion.
Figs. 16 and 17. Resting nuclei. ($4 \times \frac{1}{12}$.)
Figs. 18–23. Stages of division. (Figs. 18–21, and 23, $4 \times \frac{1}{12}$; Fig. 22, $2 \times \frac{1}{12}$.)

PLATE XII.

Teratonympha mirabilis.

Fig. 24. *T. mirabilis* var. *formosana*, whole organism. ($2 \times \frac{1}{12}$.)
Fig. 25. Anterior portion of *T. mirabilis* var. *formosana*. ($4 \times \frac{1}{12}$.)
Fig. 26. Anterior portion of *T. mirabilis* (type species). ($4 \times \frac{1}{12}$.)
Figs. 27–31. Stages of division of *T. mirabilis*. ($4 \times \frac{1}{12}$.)

PLATE XIII.

Figs. 32–38. *Microspironympha porteri.*

(In Figs. 32, 34, 36, and 37, the specimens are drawn focused on lower surface, so that the spirals appear laeotropic, though they are really dexiotropic.)

Fig. 32. Small individual. ($2 \times \frac{1}{12}$.)
Figs. 33 and 34. Large individuals. ($2 \times \frac{1}{12}$.)
Fig. 35. Diagrammatic drawing of the anterior portion.
Figs. 36 and 37. Anterior portion of large individuals. ($2 \times \frac{1}{12}$.)
Fig. 38. A late stage of division. ($2 \times \frac{1}{12}$.)

Figs. 39 and 40. *Holomastigotes elongatum* (?).

Fig. 39. Whole organism. ($4 \times 1\frac{1}{2}$.)

Fig. 40. Another specimen, showing the endoplasm and spiral ridges (really dexiotropic, but focused on lower surface). ($4 \times 1\frac{1}{2}$.)

Figs. 41 and 42. *Spirotrichonympha leidyi.*

Fig. 41. Semi-diagrammatic drawing of the organism. ($4 \times 1\frac{1}{2}$.)

Fig. 42. Whole organism. ($2 \times 1\frac{1}{2}$.)

Figs. 43–52. *Holomastigotoides hartmanni.*

(In Figs. 43, 44, 45, 47, 48, and 51, the specimens are drawn focused on lower surface, so that the spirals appear reversed (laeotropic). They are really dexiotropic.)

Fig. 43. Whole organism. ($2 \times 1\frac{1}{2}$.)

Figs. 44–46. Anterior portion. ($4 \times 1\frac{1}{2}$.)

Figs. 47–51. Stages of division. ($4 \times 1\frac{1}{2}$.)

Fig. 52. Anterior portion of a young individual, soon after division. ($4 \times 1\frac{1}{2}$.)

Fig. 53. Diagrammatic drawings showing structure of body wall of *Teratonympha* (*a*), *Holomastigotoides* (*b*), and *Holomastigotes* (*c*).

PLATE XIV.

Pyrsonympha.

(In Figs. 54, 55 and 66, the specimens are focused on lower surface, so that the spirals appear dexiotropic, though really laeotropic as in Fig. 65.)

Figs. 54 and 55. *P. grandis*, whole organisms. ($4 \times 1\frac{1}{2}$.)

Figs. 56 and 57. Anterior portion of *P. grandis*. ($4 \times 1\frac{1}{2}$.)

Figs. 58–61. Stages of division of *P. grandis*. (Figs. 58 and 60, $4 \times 1\frac{1}{2}$; Figs. 59 and 61, $2 \times 1\frac{1}{2}$.)

Figs. 62–64. Stages in degeneration of *P. grandis*. ($4 \times 1\frac{1}{2}$.)

Figs. 65 and 66. *P. modesta*; in the latter figure a screw-like form shown. ($4 \times 1\frac{1}{2}$.)

PLATE XV.

Dinenympha.

(In Figs. 68, 69, 71, 72, 79, and 81, the spirals are drawn reversely, the lower surface being focused. All spirals are really laeotropic.)

Figs. 67, 68. *D. exilis*, whole organisms. ($4 \times 1\frac{1}{2}$.)

Figs. 69, 70. *D. rugosa*, whole organisms. ($4 \times 1\frac{1}{2}$.)

Figs. 71 and 72. Middle portion of the body of *D. rugosa*, showing nuclei. More highly magnified.

Figs. 73 and 74. *D. nobilis*, whole organisms. ($4 \times 1\frac{1}{2}$.)

Fig. 75. Semi-diagrammatic drawing of the posterior portion of *D. nobilis*.

Figs. 76 and 77. *D. leidyi*, whole organisms. ($4 \times 1\frac{1}{2}$.)

Fig. 78. *D. parva*, whole organism. ($4 \times 1\frac{1}{2}$.)

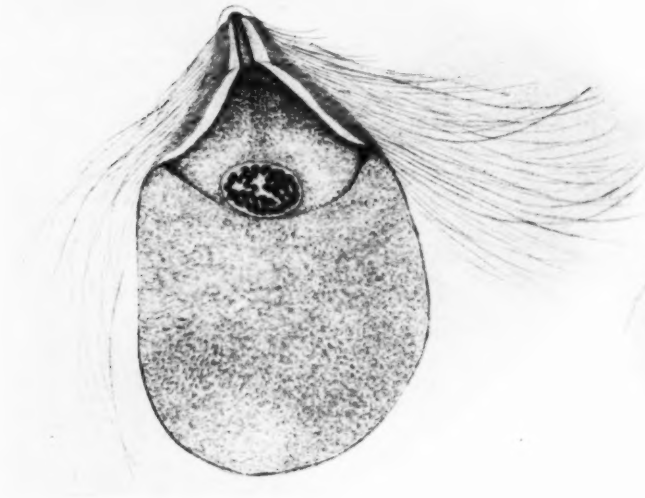
Fig. 79. *D. porteri*, type 1. ($4 \times 1\frac{1}{2}$.)

Fig. 80. *D. porteri*, type 2. ($4 \times 1\frac{1}{2}$.)

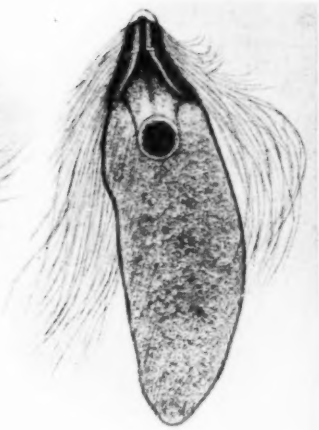
Fig. 81. *D. porteri*, type 3. ($4 \times 1\frac{1}{2}$.)

Fig. 82. Semi-diagrammatic drawing of the posterior end of *D. porteri*, type 1.

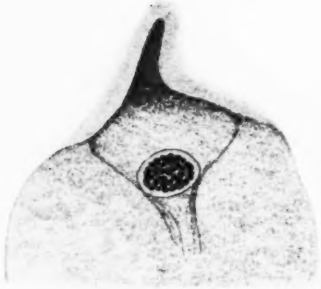
Figs. 83 and 84. Stages of division of *D. porteri*. ($4 \times 1\frac{1}{2}$.)



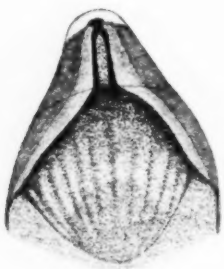
1



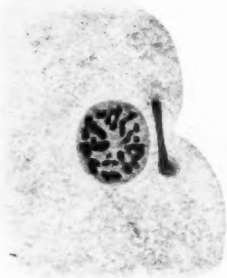
5



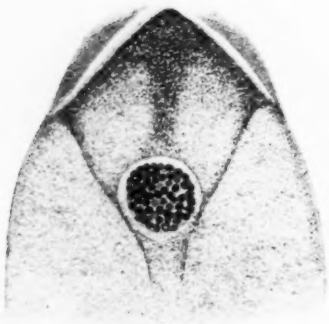
2



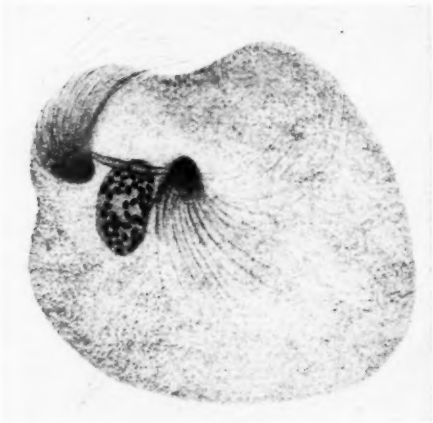
4



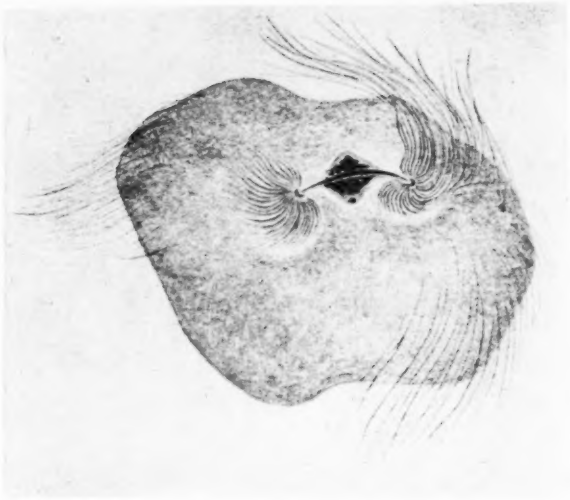
6



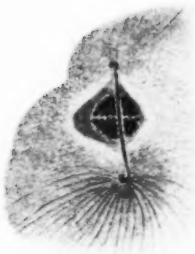
3



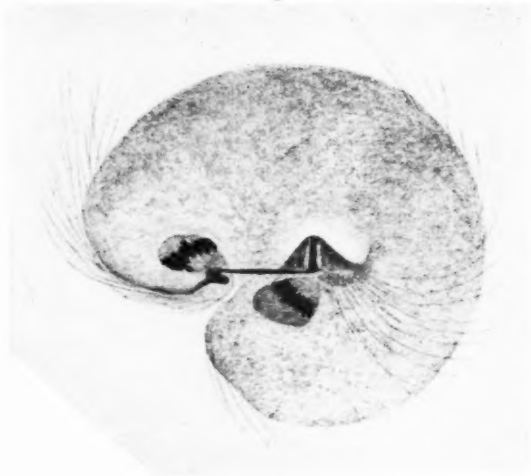
7



9

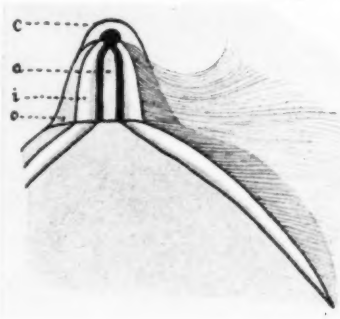


10

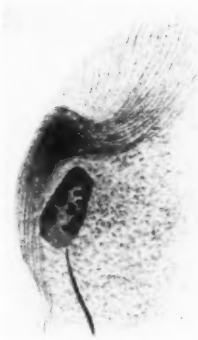


11

8

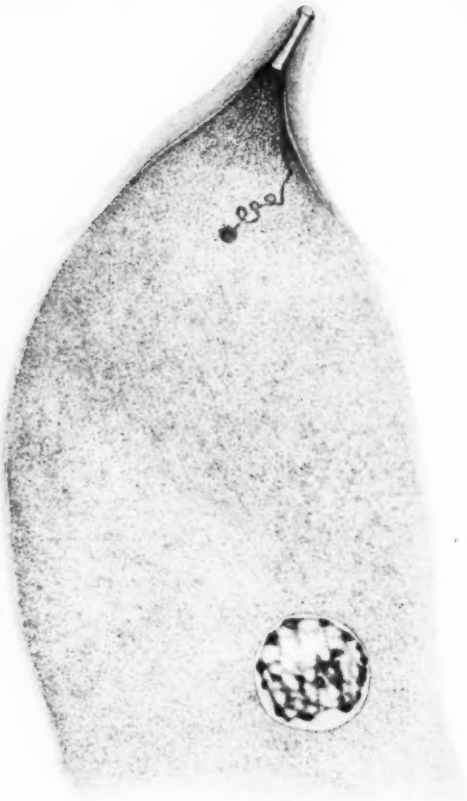


13

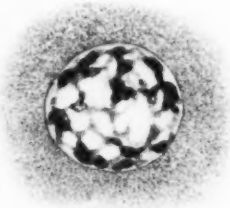


12

14

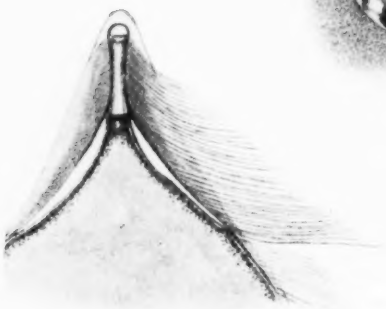


18



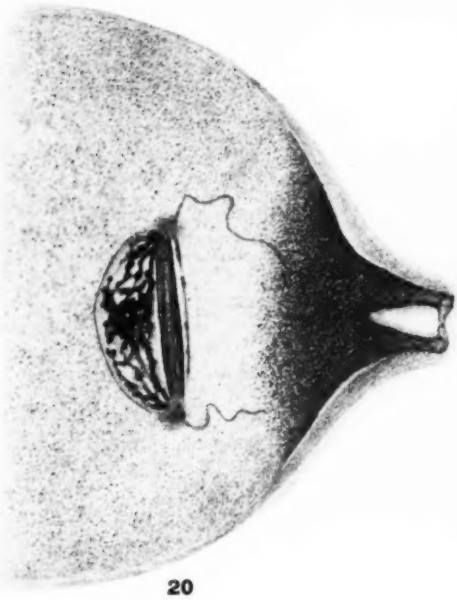
16

17

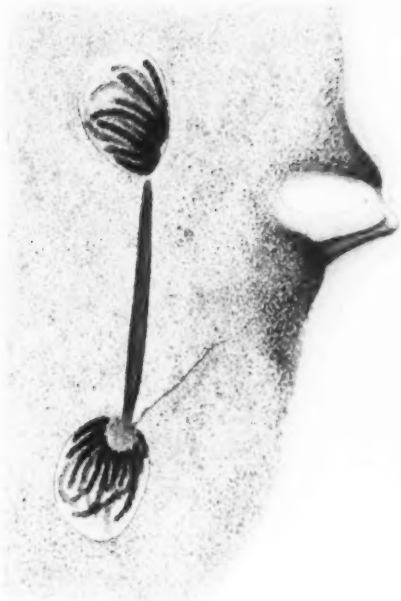


15

23

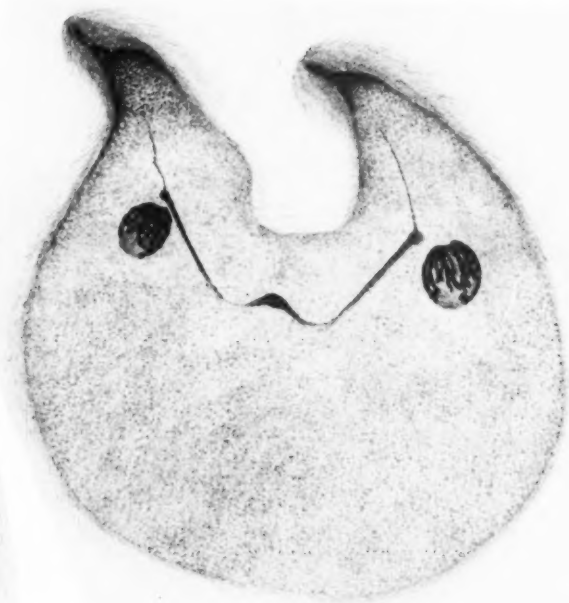


20

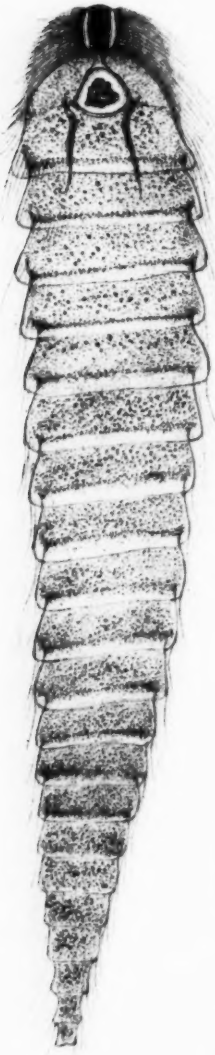


21

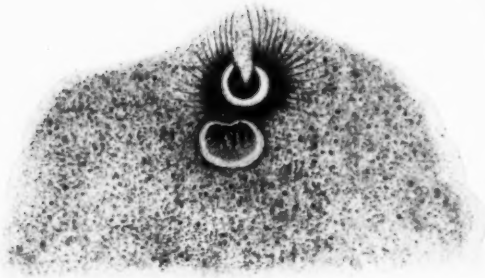
19



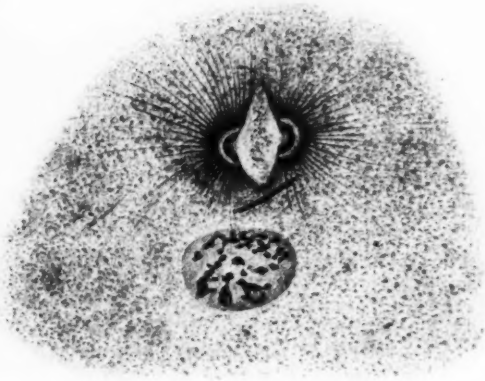
22



24

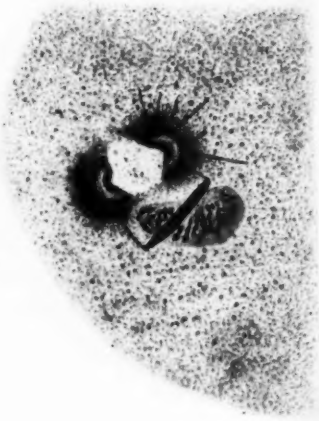


31



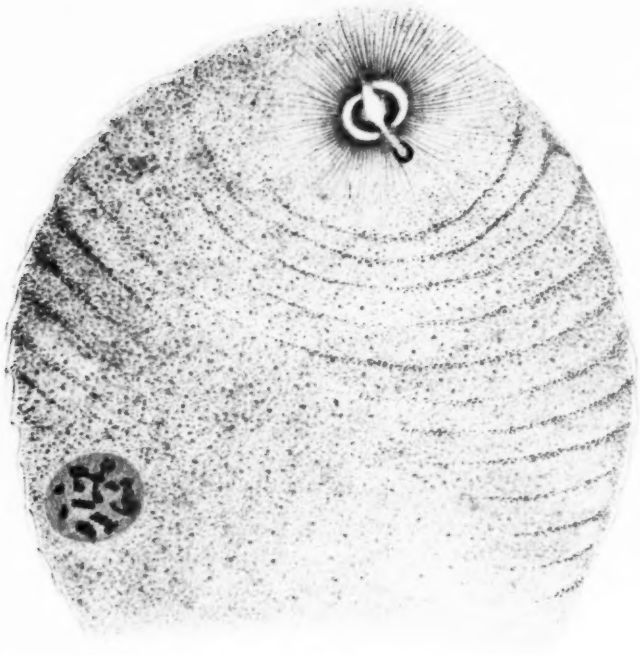
28

25

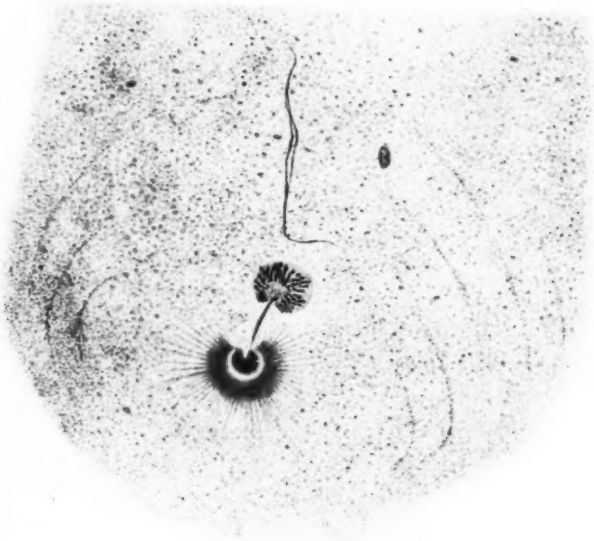


29

26



27



30

32

33

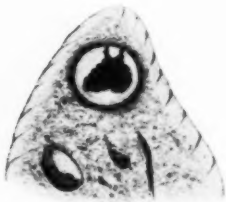
34

35

36

38

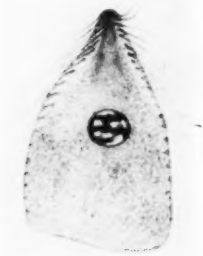
37



52

39

40

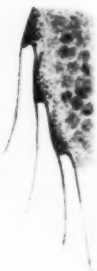


41

42

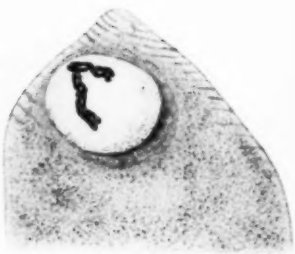
a

b



c

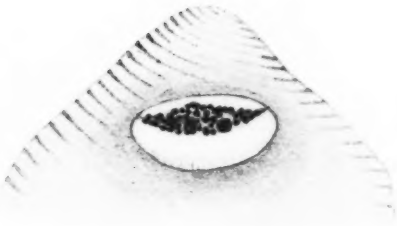
53



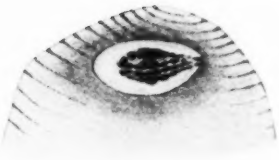
51

43

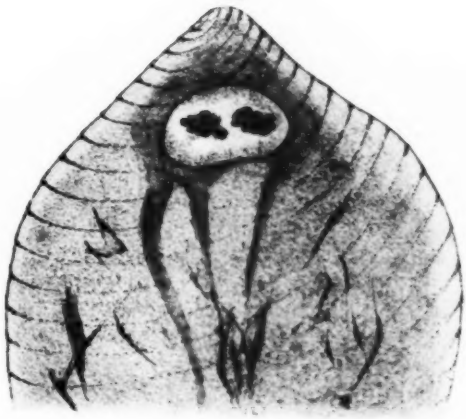
44



45



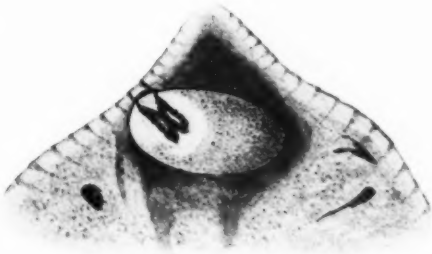
47



46

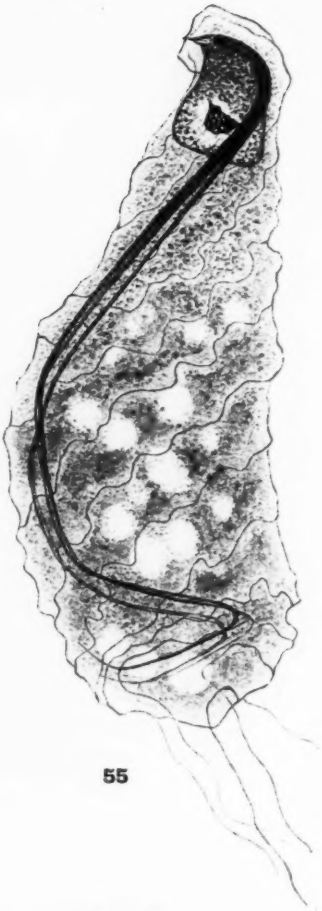
48

49

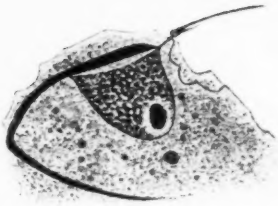


50

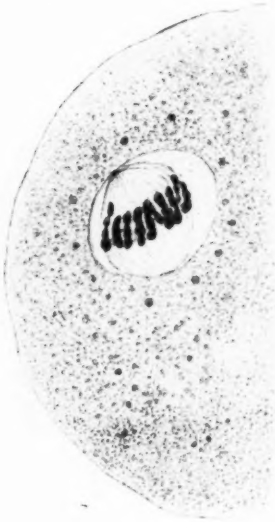
54



55



56



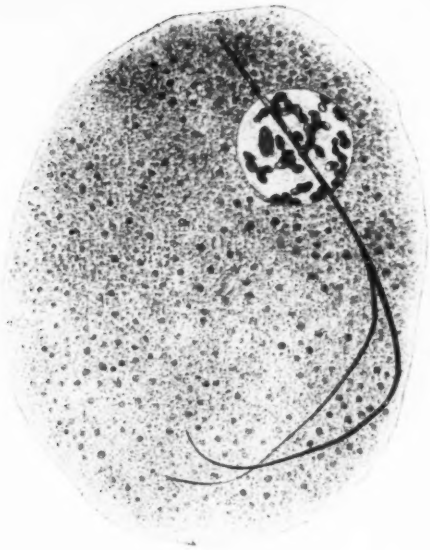
60

65

66

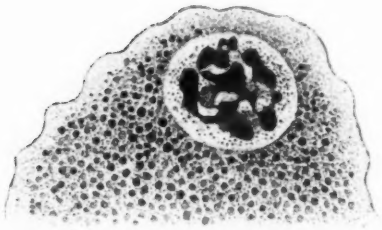
57

61

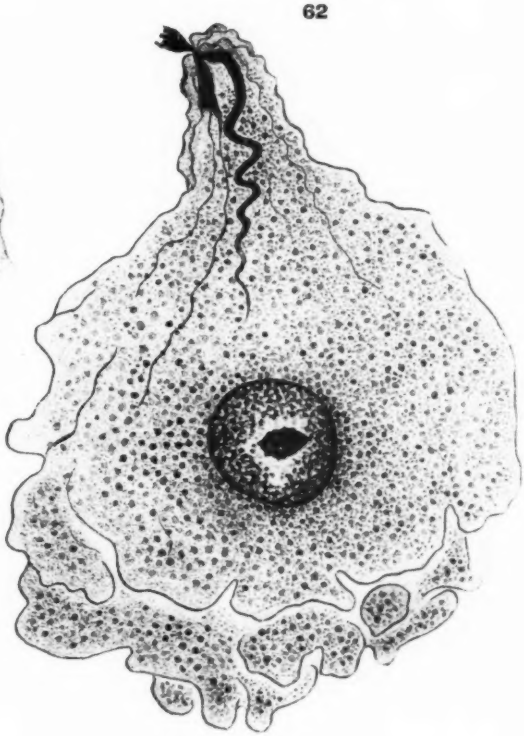


58

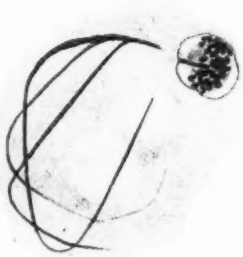
62



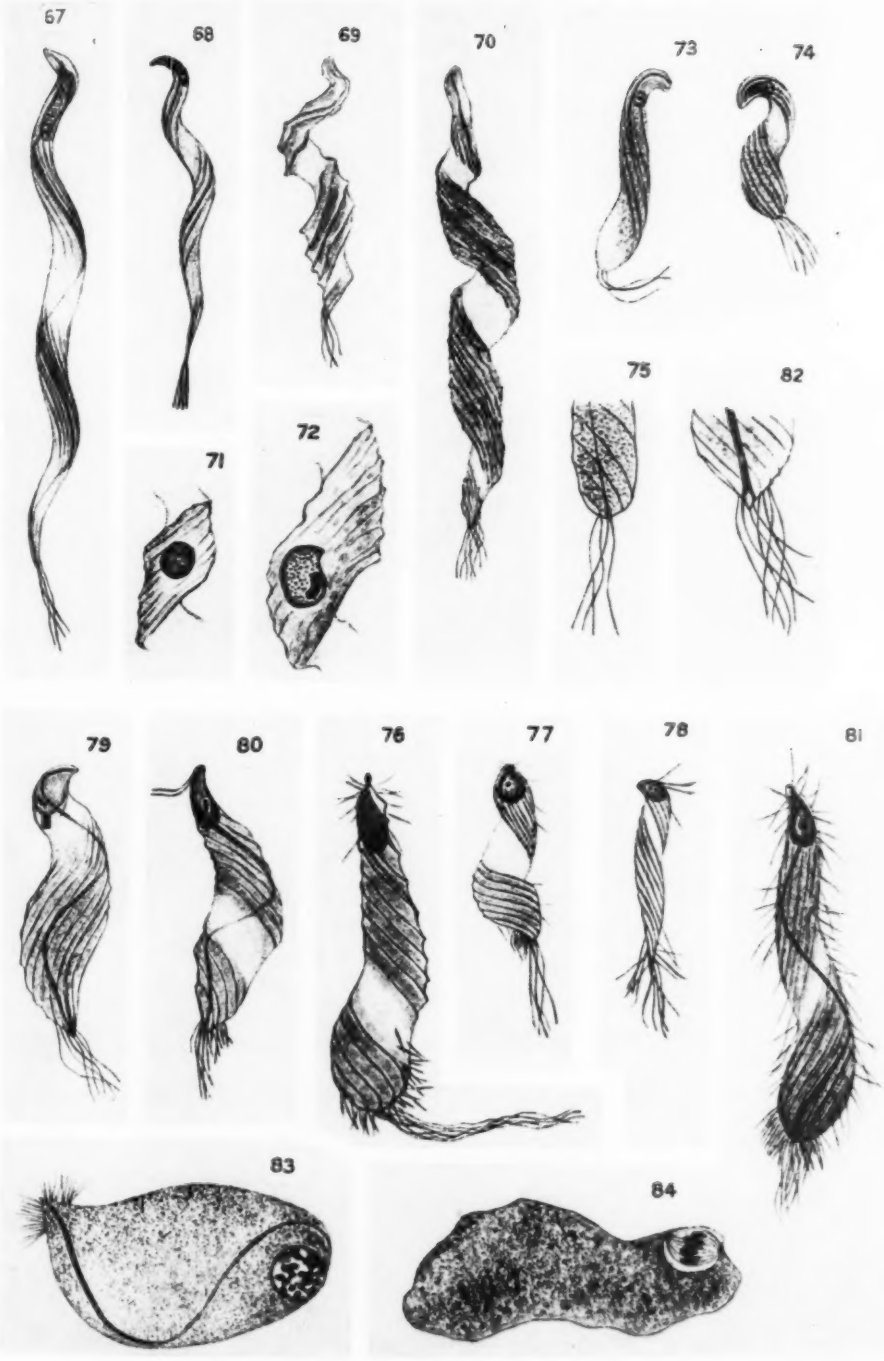
64



63



59


67
68
69
70
73
74
71
72
75
82
79
80
76
77
78
81
83
84